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# THE ACTION OF ALTERNATING CURRENTS UPON THE ELECTRICAL EXCITABILITY OF NERVE

J. REBOUL AND A. ROSENBLUETH

*From the Department of Physiology in the Harvard Medical School*

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That alternating currents (a.c.) may modify the electrical excitability of nerve not only at the region of application but also in neighboring segments was shown by Wedensky in 1903. In a study of some effects of a.c. on nerve, observations have been made which confirm and extend Wedensky's classical report. The present paper is concerned with these observations.

METHOD. The general procedure and the apparatus employed were indicated in a preceding communication (Rosenblueth and Reboul, 1939). Only a brief description, therefore, is necessary here.

Cats were used, under dial anesthesia (Ciba 0.7 cc. per kgm. intraperitoneally). The majority of the observations were made recording the contractions of the gastrocnemius-soleus muscle as indicators of the responses of the popliteal nerve to the test stimuli delivered. The spike potentials of the A fibers of the excised peroneal nerve were also used to confirm the observations made on the nerve-muscle preparations, thus excluding muscular phenomena.

From 6 to 10 chlorided silver-wire electrodes were placed on the nerves. The following conventions will be used with regard to these electrodes. When the terms near and far are employed, the muscle will be taken as the reference point. If numbers are used for the electrodes, increasing figures will denote nearness to the muscle.

The study consisted essentially in the investigation of the changes in the responses to submaximal test shocks delivered to a pair of electrodes when a.c. was sent to the nerve through the same or other electrodes, farther from the muscle or nearer to it. Usually two regions of the nerve were tested alternatively in each observation. The position of these two regions with respect to the a.c. varied in different experiments for different purposes.

The a.c. electrodes divide the nerve into three segments: an interpolar region, between the two electrodes, and two extrapolar regions, far and near. One of the test electrode-pairs was usually in the near extrapolar region. The location of the other test pair and the characteristics of the shocks delivered to it depended upon the information or the control desired.

The test shocks were condenser discharges of voltage and capacity adequate for the effect required, submaximal or maximal. For maximal responses the stimuli were 3 to 4 times supramaximal. Two completely independent circuits were used when the test shocks were delivered to two regions of the nerve. The cathodes were proximal to the muscle.

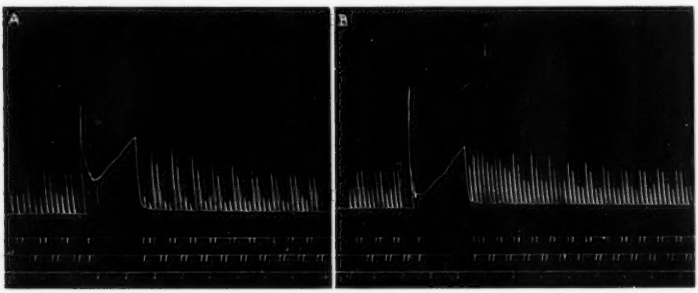


Fig. 1. Opposite effects of different intensities of a.c. on the electrical excitability of nerve. Three pairs of electrodes applied to the popliteal nerve, far, middle and near with respect to the gastrocnemius-soleus muscle. Upper signal: submaximal condenser discharges applied to middle electrodes. Middle signal: maximal discharges applied to near electrodes.

A. Between the times marked by the simultaneous upper and middle signals a.c. (frequency 1,000, voltage 3) applied to far electrodes.

B. Same, but voltage 17. In this and the succeeding figures the time signal indicates 5-sec. intervals.

RESULTS. I. *The influence of the intensity, frequency and duration of the a.c.* At any given point of the nerve, interpolar, polar or extrapolar (within about 20 mm. from the pole), the characteristics of the a.c. applied determined the changes of electrical excitability produced.

With constant frequency and period of application, a.c. of various intensities resulted in striking reversible changes of electrical excitability. Weak currents produced a decrease, whereas stronger currents led to an increase usually followed by a late decrease of excitability, with a slow recovery to normal. In exceptional cases currents of intermediate intensity resulted in a slight immediate decrease followed by a delayed increase of excitability. Very strong currents, finally, produced an initial decrease

of the test responses, then an increase (see fig. 6), and lastly sometimes a delayed decrease.

In figure 1 is illustrated a typical instance of the opposite effects of weak and strong currents. Submaximal test shocks were delivered in the near extrapolar region, close to the lower a.c. pole. Maximal test shocks were also applied in the same extrapolar region, but nearer to the muscle. The responses to the latter maximal test shocks control for post-tetanic changes of the muscular twitches. These twitches show only a slight post-tetanic increment in both A and B. The marked changes in the responses to the submaximal shocks were therefore due to changes in the electrical excitability of the nerve. A typical case of an initial increase followed by a delayed decrease of excitability upon application of strong a.c. is illustrated in figure 2.

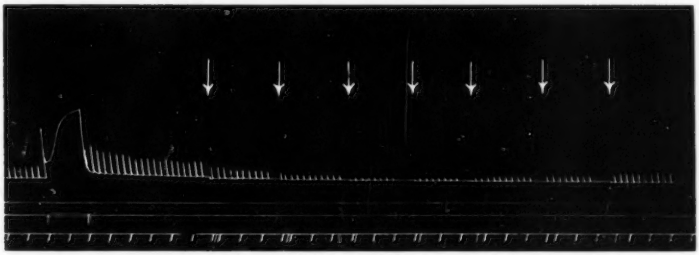

Fig. 2. Delayed decrease of excitability. Two pairs of electrodes on the popliteal nerve. Submaximal condenser discharges delivered throughout to the pair near the muscle at the rate indicated by the twitches. Between signals a.c. (frequency 16,000, voltage 17) applied to the far electrodes. The arrows denote intervals of 2, 3, 2, 3, 4, 3 and 6 minutes.

When different frequencies of a.c. were compared, their effects upon electrical excitability were found qualitatively similar. A quantitative difference was apparent, however, in that for higher frequencies a higher critical intensity separated the depressing from the enhancing actions upon excitability.

The length of time during which a.c. of a given frequency and intensity was applied influenced the sign, magnitude and duration of the changes of excitability. Figure 3 illustrates the phenomenon. With a weak current (A) a brief period of application was ineffective, while a longer period resulted in a decrease of excitability. With a medium intensity (B and C) brief applications of a.c. gave an increase, whereas long periods resulted in a decrease of excitability. With strong a.c. (D) prolonging the duration of delivery resulted in greater augmentations of excitability. It appears, therefore, that greater duration of a.c. emphasizes both the decrease and the increase of excitability, but the former more than the latter.

II. *The differences between the interpolar, polar and extrapolar regions.* All the changes of excitability reported in section I could be obtained in any of the regions of the nerve separated by the a.c. poles, up to distances of about 20 mm. from the poles in the extrapolar regions. When different points of the nerve were tested after a given application of a.c., differences were observed, both qualitative and quantitative, with which this section is concerned. These results may be summarized by two general statements: *a*, the effects were maximal at the poles and decreased progressively

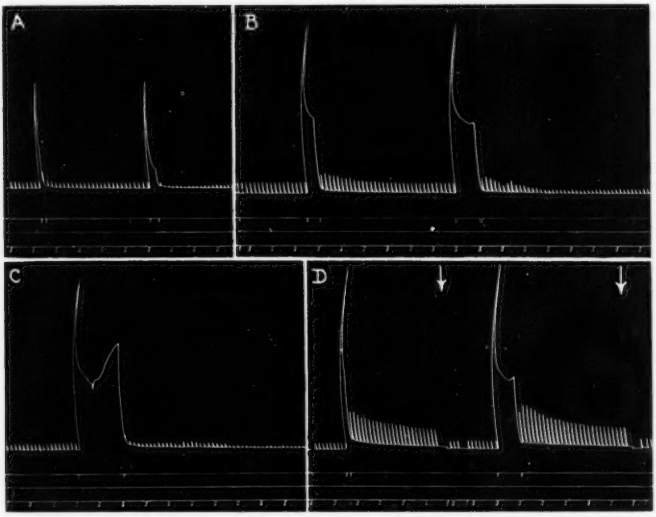


Fig. 3. Influence of the length of the periods of application of a.c. on the changes of electrical excitability. Four electrodes—1, far, to 4, near to the muscle—applied to the popliteal nerve. Submaximal condenser discharges delivered throughout to electrodes 2 and 3. Between signals a.c. (frequency 500) applied to electrodes 1 and 4.

A, voltage of a.c. 0.3. B, voltage 9.5. C, same as B. D, voltage 17. The arrows indicate 1-minute intervals.

at points more and more distant from these poles; *b*, the critical intensity of a.c. separating the depressing from the enhancing actions upon excitability was lowest at the poles and increased progressively with the distance from these poles.

Figure 4 illustrates the data summarized in statement *a*. A given application of relatively intense a.c. was repeated at intervals of about 5 minutes, and its action upon the electrical excitability of different points in the nerve was observed. The increasing action was maximal at the

poles and decreased for points in the interpolar or extrapolar regions distant from the poles.

The statement *b* made above leads to the expectation that a given application of a.c. may result in increased excitability at some point of the nerve near one of the poles and simultaneously a decreased excitability

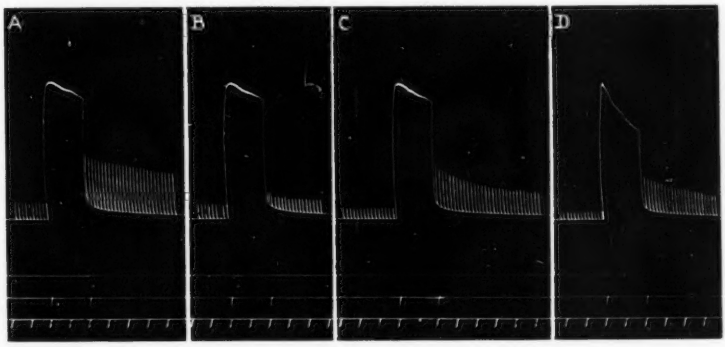


Fig. 4. Comparison of the interpolar, polar and extrapolar regions of the nerve. Seven electrodes (1, far, to 7, near the muscle) applied to the popliteal nerve at distances of about 10 mm. Between signals a.c., frequency 40,000, voltage 7, applied to electrodes 2 and 6. Submaximal condenser discharges delivered as follows: A, to electrodes 1 and 2; B, 3 and 4; C, 5 and 6; D, 6 and 7.

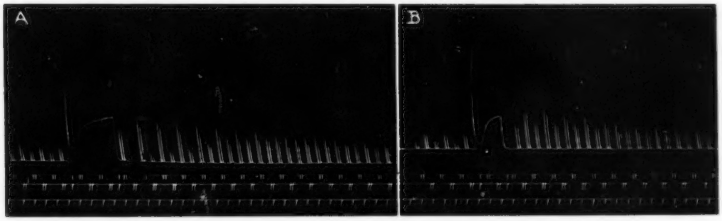


Fig. 5. Influence of the distance from the a.c. pole on the changes of excitability. Six electrodes (1 to 6) applied to the popliteal nerve at distances of about 10 mm. Upper signal: submaximal condenser discharges delivered to electrodes 3 and 4. Middle signal: similar shocks to electrodes 5 and 6. At the time marked by the simultaneous upper and middle signals a.c., frequency 500, applied to electrodes 1 and 2.

A. Voltage 10; B, 20.

at some point further away from the pole. Figure 5 shows that this expectation was fulfilled. When very strong currents were used the test responses from the extrapolar region were depressed in the immediate vicinity of the pole, further away they were increased (see Wedensky, 1903), and still further they were decreased.

III. *Changes brought about by repeated application of a.c.* When a.c. of constant or variable intensity was applied repeatedly at relatively short time-intervals (1 to 5 min.) the results from a given intensity were not always the same, even if the effects of the previous application had apparently subsided. A common example of this influence of previous treatment by a.c. on subsequent observations was the following. A weak current was delivered, which decreased the electrical excitability at the region of the nerve tested. A strong current, with its usual enhancing effect was then applied. If the original weak current was again tested within a few minutes, it was found to have no decreasing influence upon the excitability, or even an enhancing action.

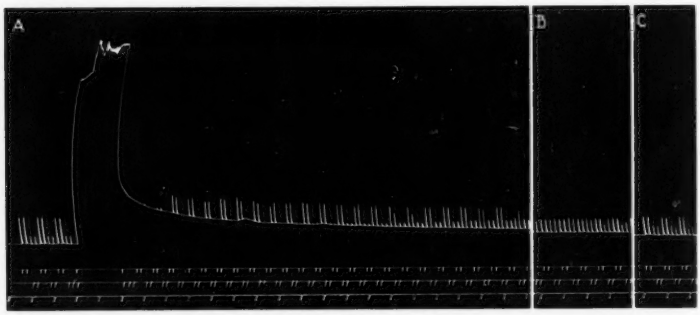


Fig. 6. Block and increased electrical excitability. Three pairs of electrodes on popliteal nerve, far, middle and near to the muscle. Upper signal: maximal condenser discharges delivered to the far electrodes. Middle signal: submaximal discharges to the near electrodes. The tetanic contraction of the muscle indicates the application of a.c. (frequency 16,000, voltage 54) to the middle electrodes. Between A and B, 1 min. and between B and C, 2 min. intervals.

IV. *The deblocking and blocking effects of a.c. and the changes of excitability.* In the preceding communication (Rosenblueth and Reboul, 1939) it was shown that a.c. exerts both a deblocking and a blocking influence upon nerves, and that with weak currents the deblocking influence usually preponderates, whereas strong currents lead to block. The present observations indicate similarly two opposite influences upon the excitability, exhibited by different intensities. It was considered important to determine whether any correlation would be present, for instance, if a current which deblocks would invariably lead to decreased excitability.

The tests were made in nerves partially blocked by previous applications of strong a.c., so that both deblocking and blocking of some nerve fibers could be apparent. The test shocks for block or deblock were maximal and were delivered in the far extrapolar region. The test shocks for

changes in excitability were as usual submaximal and were applied preferably with the cathode at the near pole of the a.c. or at some region in its close vicinity.

The experiments failed to establish the correlation in question. Intensities of current too weak to block any nerve fibers could be strong enough to increase the electrical excitability (fig. 4). A deblocking effect accompanied by increased excitability is illustrated in figure 7.

When the intensity of the a.c. was sufficiently high for block the changes of electrical excitability in the blocked region could of course not be detected, because the nerve responses, if present, would not be conducted. Certain observations, however, suggest that in these conditions there may be an increased electrical excitability. In figure 6 the a.c. blocked all the fibers in the nerve for some time. The test responses from the near extrapolar region were first decreased, but later increased. These results may be interpreted by assuming that the block extended to the region of the near test electrodes. When this block subsided the increased excitability produced by the current became apparent. If this explanation is accepted, whether or not the increased electrical excitability will be detected will depend on which of the two processes, block and hyperexcitability, will subside first at the point tested. This would explain why such hyperexcitability was not invariably found in the experiments.

To summarize, the general statement may be made that the critical intensity separating the depression from the enhancement of electrical excitability was lower than the intensity separating deblocking action from block.

V. *The changes of excitability revealed by the muscular responses to a.c.* In the majority of the observations the a.c. was intense enough to stimulate the nerve. The muscle contracted, therefore, during the period of application. These muscular responses afford a basis for studying the changes of excitability during the passage of a.c.

The results from high frequencies (above 3,000 per sec.) are easier to evaluate than the others. They will be considered first and the observations with lower frequencies will be described afterwards. In figure 8 are illustrated typical responses of a muscle to high frequency stimulation with various intensities. As the intensity was increased the responses first augmented and were better sustained during the period of application of a.c. (cf. A and B). Further intensification of the current, however, led to decreased and less sustained muscular contractions. The myograms resembled then those which result from Wedensky inhibition. That Wedensky inhibition played no rôle in these observations was shown, however, by the ability of test shocks applied in the near extrapolar region to elicit fully maximal twitches.

It may be concluded, therefore, that prolonged applications of high

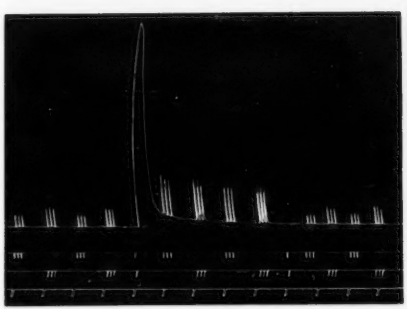

Fig. 7

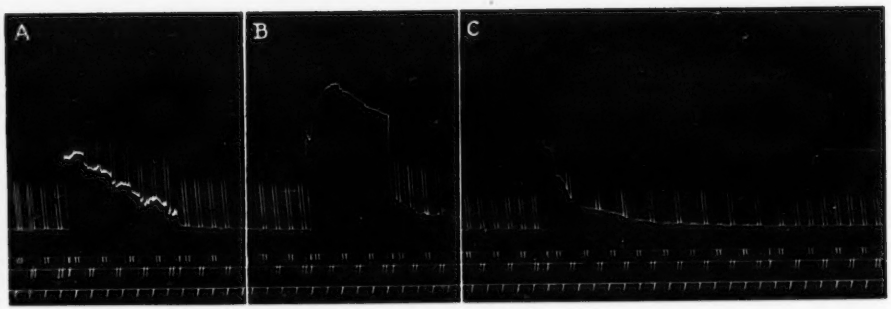


Fig. 8

Fig. 7. Deblocking and increased excitability. Five electrodes (1 to 5) on popliteal nerve at distances of about 14 mm. A large fraction of the fibers in the nerve was blocked because of several previous applications of strong a.c. to electrodes 3 and 5. Upper signal: maximal condenser discharges delivered to electrodes 1 and 2. Middle signal: submaximal discharges to electrodes 5 and 6. During the time marked by the simultaneous upper and middle signals, a.c. (frequency 40,000, voltage 10) applied to electrodes 3 and 5.

Fig. 8. Muscular responses to high frequency a.c. Three pairs of electrodes on the popliteal nerve. Upper signal: maximal condenser discharges delivered to far electrodes. Middle signal: similar shocks to near electrodes. During the time marked by the simultaneous upper and middle signals a.c. (frequency 40,000) applied to the middle electrodes. The voltages were as follows: A, 2; B, 12; C, 30.

frequency a.c. cause a decrease of electrical excitability more marked for weak or strong currents than for currents of medium intensity. This conclusion is in accord with the data on the influence of duration of a.c. on the changes of excitability reported in section I (fig. 3).

With frequencies between 300 and 2,000, Wedensky inhibition invariably occurred (see figs. 1, 3 and 5). The neuromuscular phenomena were then too complex for a ready interpretation of the changes taking place at the nerve. Frequencies lower than 100, on the other hand, are too slow for the production of Wedensky inhibition. With such slow frequencies effects similar to those described for high frequencies were often encountered—i.e., the muscular contractions could decrease when the intensity was increased. The phenomenon is therefore probably present at all frequencies.

Discussion. The data reported in sections I and II indicate that applications of a.c. originate at least two different processes with opposite effects on the electrical excitability of nerve. The processes are maximal at the site of contact of the a.c. electrodes and extend to the neighboring regions of the nerve, declining in magnitude as the distance from the a.c. poles increases.

A theory based on the development of a single process would fail to account for the facts satisfactorily. The unlikely assumption would have to be made that increasing the intensity—i.e., increasing the magnitude of the process—would first depress and later enhance the excitability. Furthermore, during the subsidence of the process a depression would always follow the phase of increased excitability, and the optimum depression should always be met. Although the cases of delayed depression were common (fig. 2) they were not invariably found. An intensity, frequency and duration of a.c. could always be determined which led to pure enhancement, not followed by depression.

If, for the preceding reasons, the existence of two different processes is accepted, their independence in time and in space is probable. Independence in time—i.e., different rates of production and of subsidence—would explain the various sequences of effects encountered (cf. figs. 1, 2, 3 and 4). Independence in space—i.e., different extent along the nerve—would explain the differences, both qualitative and quantitative, which were found in different regions of the nerve (fig. 5).

The possibility of recording opposite effects in neighboring segments of the nerve indicates that the two actions of a.c. upon excitability are direct effects of the current, not indirect consequences of the nervous activity stimulated during the period of application.

According to Hill's (1936) theory of electrical stimulation a current produces two independent effects on nerve. At the cathode the "local potential" rises, while the threshold increases because of the process of

"accommodation." At the anode the opposite changes would take place. Since the effects at the anode and cathode are assumed equal and opposite in sign, when a.c. is applied any given half cycle would exactly cancel the action of the preceding half cycle (Hill, Katz and Solandt, 1936). The final action would therefore depend exclusively on the phase of the current at which it was stopped, and would be independent of the time of application. It is obvious, therefore, that the concepts of local potential and accommodation do not account for the data in sections I and II.

Applications of a.c. induce changes in nerve polarization (Wedensky, 1903). It might be thought that these electrotonic changes could account for the variations in electrical excitability. A report of the changes of polarization encountered in the present study will be published later. It may be stated here, however, that the changes of excitability could not be correlated with the changes of polarization. For instance, the same degree of negativity of the a.c. region with respect to a distant segment of the nerve was attended sometimes by decreased, sometimes by increased excitability.

The changes of excitability during the application of a.c. revealed by the responses to the a.c. itself (section V, fig. 8) have been recently studied by Cattell and Gerard (1935), Bugnard and Hill (1935), and Cattell and Grundfest (1935). The decrease of excitability demonstrated by the absence of responses after some time of application of a.c. was attributed to anelectrotonus, to relative refractoriness and to post-cathodal depression, respectively. These observations were mainly made with just threshold, or slightly above threshold a.c. Whether one or several of these explanations is accepted it would be expected that as the high-frequency a.c. is intensified the decrease of excitability should become more prominent (cf. Bugnard and Hill, 1935). Figure 8 shows, however, that such is not the case. There is an optimum intensity at which the muscular responses are better sustained, indicating that the nerve excitability is little modified by the current. That the decline in the muscular contraction, when present, was not due to Wedensky inhibition was demonstrated by the responses to the control stimuli applied proximally to the muscle. Indeed, in the present observations instances of true Wedensky inhibition were only exceptionally encountered with frequencies higher than about 4,000 per sec. at any of the intensities used. In the absence of Wedensky inhibition the muscular contractions are probably the best indicator available of nervous activity in response to high-frequency stimulation.

The explanations mentioned may not account for the existence of an optimum intensity because they postulate only one factor: decreased excitability. If the two independent effects assumed previously are brought to bear on the problem the results may be readily interpreted.

With weak intensities the process responsible for depressed excitability would preponderate. As the intensity is increased, the opposite enhancing process would become more and more prominent, and would account for the existence of the optimum intensity. When the intensity reaches a certain level, however, block of some of the fibers will begin to appear. This block probably extends beyond the a.c. poles. The rapid decline with strong currents would therefore be due to an increasing blocking action.

If this explanation were adequate, block should always be demonstrable with intensities of current greater than the optimum. This inference was supported by the data (see fig. 8C).

In the preceding paper (Rosenblueth and Reboul, 1939) the inference was drawn that a.c. exerts two independent opposite influences on nerve conduction, one deblocking, the other blocking nerve impulses. The present data lead to a similar conclusion with respect to electrical excitability; there are two influences, one depressing, the other enhancing the excitability. In section IV it was shown that the facts do not reveal any correlation between these four actions. The complexity of the data emphasizes the complexity of nerve; it seems likely that no simple theory will account satisfactorily for the facts.

## SUMMARY

Applications of weak a.c. to a nerve decrease locally the electrical excitability (fig. 1A). Stronger currents lead, on the contrary, to increased excitability (fig. 1B) which may be followed by a delayed decrease (fig. 2).

The frequency of the a.c. (section I) and the length of application (fig. 3) influence the results obtained.

The effects are maximal at the poles and decrease with the distance from these poles (fig. 4). Different segments of the nerve may show opposite changes of excitability (fig. 5).

It is concluded that a.c. exerts two independent opposite effects on excitability. These effects are not correlated with the effects of a.c. upon nerve conduction (section IV; figs. 6 and 7).

The influence of the intensity of high frequency a.c. on the muscular responses to the a.c. is described (fig. 8). These results support the previous conclusions (p. 214).

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# EVIDENCE OF VASODILATOR INNERVATION IN THE PARIETAL CORTEX OF THE CAT

HENRY S. FORBES, CARL F. SCHMIDT AND GLADYS I. NASON

*From the Marine Biological Laboratory, Woods Hole, Mass.*

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We already have physiological evidence that arteries of the brain in certain regions are supplied with vasomotor nerves. The existence of constrictor nerves has been confirmed by several different experimental methods (1). Evidence for dilator nerves has been obtained chiefly by one method—the measurement of large arteries on the surface of the brain (2).

In order to get more data concerning this dilator mechanism further experiments were carried out, using an experimental method different from the one previously employed. The present method has been used successfully by Schmidt for studying vasoconstrictor nerve activity in various parts of the brain. By measuring changes in blood-flow through the minute vessels within the cerebral cortex this method serves as a check on the earlier observations (2).

METHOD. The thermoelectric method described by Schmidt and Pierson (3) was used to record changes in blood-flow by means of a thermocouple inserted 2 or 3 mm. into the parietal cortex of cats anesthetized with "Dial Liquid with Urethane"[1] (0.5 cc. per kgm.) intraperitoneally. The ipsilateral facial nerve in its bony canal was exposed and cut centrally and peripherally to the origin of the great superficial petrosal nerve, which remained intact. Open electrodes (usually bipolar) were applied to the exposed facial nerve just proximal to the geniculate ganglion. The electrodes were then clamped in place. Stimulation (faradic) ensued without further manipulation. Graphic records were obtained by means of a photokymograph. Details of this technique have been described in previous papers (2) (4).

*Results.* Nine cats and one monkey were operated on as described. The sensitivity of the thermoelectric apparatus to changes in blood-flow about the active junction in the brain was tested in each animal by measuring the deflection of the galvanometer when the carotid arteries were clamped.

[1] The dial was supplied by Ciba Company, Inc., Summit, N. J.

With three cats the sensitivity of the preparation proved to be inadequate and these experiments were discarded.

In three other animals (two cats and a monkey) the preparation showed fair sensitivity. On stimulation of the geniculate ganglion there was some slight indication of increased blood-flow in the cortex. This occurred only one or more times during several trials. The results were considered unsatisfactory since the changes were small and inconsistent.

In the remaining four cats the sensitivity of the preparation was good, judging by the response to carotid occlusion (fig. 1). In all of these ani-

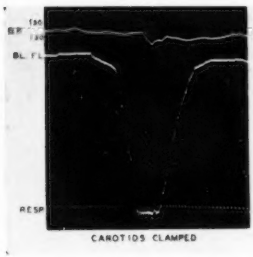


Fig. 1

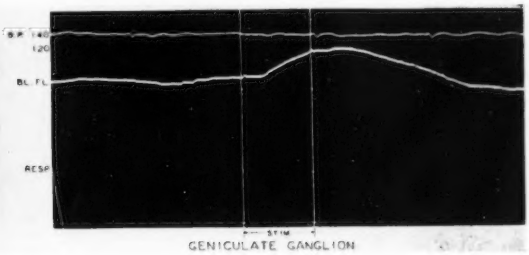


Fig. 2

Fig. 1. Effect of carotid occlusion, illustrating the degree of sensitiveness of this preparation. Both common carotid arteries were clamped for a period of 15 seconds. The indicated blood-flow in the cerebral cortex fell promptly 12 cm.

In this figure and in the following ones *B.P.* refers to blood pressure in the femoral artery; *Bl.Fl.* refers to changes in rate of blood flow in the parietal cortex as indicated by the thermoelectric apparatus and recorded by a manually operated lever following the movements of the galvanometer image. *Resp.* refers to changes in respiration recorded from the trachea by a Brodie bellows recorder. The time marker records five second intervals.

Fig. 2. (Same cat as fig. 1.) Stimulation of the facial nerve at the geniculate ganglion. (Coil distance, 13 cm. — bi-polar silver electrodes fixed in situ.) The indicated flow (*Bl.Fl.*) rose 2 cm. The two vertical lines indicate the period of stimulation. The latent period was 10 seconds, and recovery occurred in 65 seconds after the stimulation. Similar results were obtained in 5 other stimulations in this animal.

mals the blood-flow in the parietal cortex increased on repeated stimulation of the facial nerve at the geniculate ganglion.[2] There were some variations in response owing to variations in strength of stimulation, method of applying the electrodes and general condition of the animal; but on the whole the amount of response and the time relations—especially the latent period between the start of the stimulation and the beginning of the re-

[2] In certain instances no reaction was obtained until the facial nerve was dissected out closer to the geniculate ganglion. When the electrodes were replaced close to the ganglion the typical reaction occurred with every stimulation.

sponse—were consistent (fig. 2). In one of these cats, in addition to measurements of blood-flow, observations were made of the pial arteries through a cranial window.[3] In this animal the dilator response of a large artery on the surface corresponded closely to the response of the arterioles within the cortex—as indicated by the period of increased flow. These measurements, made in the same animal by two different methods, show a similar latent period and duration of response (figs. 3 and 4).

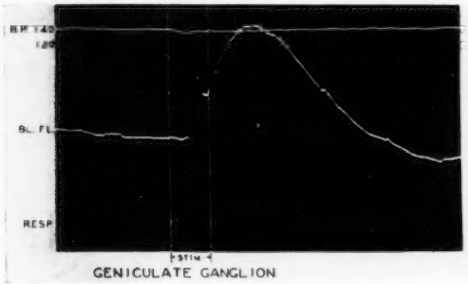


Fig. 3

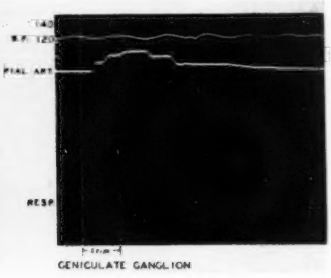


Fig. 4

Fig. 3. Stimulation of the facial nerve at the geniculate ganglion. (Coil distance 13 cm. at an angle of 30° — bi-polar electrodes fixed in situ.) The indicated flow (*Bl.Fl.*) rose 7.5 cm. The latent period of this response was 8 seconds; recovery occurred in 65 seconds after the stimulation. Similar results were obtained in 5 other stimulations in this animal.

This record is from another cat in which the sensitivity of the preparation was good (blood-flow fell 37 cm. when both carotids were clamped for 30 seconds).

Fig. 4. This record was taken from the same cat as the record shown in figure 3 (98 minutes later). Stimulation of the facial nerve at the geniculate ganglion. (Coil distance 13 cm. — bi-polar electrodes.) Pial artery measurements were made by microscope through a cranial window and recorded by a manually operated lever. The artery, 195 microns in diameter, dilated 17 per cent. The latent period of this response was 5 seconds; recovery occurred in 65 seconds—though the reproduction of this record does not clearly show this. Both observations were made in the same region of the parietal cortex, the thermocouple being removed and the window inserted at the same site.

The record of the pial artery represents changes in diameter and hence in calibre (since the artery was not flattened by the window) which were observed in a relatively large artery on the brain surface.

*Comment.* Considering the difficulties of the operation, the consistency of these results seems to us of distinct value in support of the thesis that true vasodilator innervation of cerebral vessels is present in mammals.

Precautions were taken to exclude accidental variables. We found that

[3] The normal intracranial pressure relations were preserved and the surface of the brain was protected from drying, sudden temperature changes and air currents, since it was bathed in its normal cerebrospinal fluid and enclosed by a rigid covering.

on stimulating the geniculate ganglion an increase in blood-flow occurred in the parietal cortex without change in blood pressure or in respiration, and in the absence of muscular twitchings; also, stimulation of the muscles of the head and neck caused no change in cortical blood-flow.

The objection by Wiggers that the observed dilatation may result not from activity of vasodilator nerves but from passive distention of the larger vessels on the surface of the brain (due to constriction of arterioles within its substance) is difficult to answer positively. It can be said, however, that no satisfactory evidence has been offered in support of his theory. Wiggers cites the results of experiments by himself in which dogs' brains, removed from the skulls, were perfused with a rhythmic stream of blood — Locke's solution. "In four experiments," he says, "epinephrine unquestionably decreased the venous outflow and raised chiefly the diastolic pressure, but enlargened the pial vessels" (5). No actual measurements of vessel calibre however were made, nor was it stated whether arteries or veins appeared to be wider. Furthermore the brain was no longer within the cranium. The pressure relations therefore were entirely abnormal; also the vessels on the surface were of necessity exposed to trauma, asphyxia and temperature changes. Any one of these conditions is capable of causing extreme changes in vessel calibre. There is little analogy between the conditions of his experiments and of ours, in which the normal intracranial pressure relations were retained.

Also, it seems improbable that stimulation of parasympathetic nerves should cause constriction in the depths of the brain, and we now have evidence that within the parietal cortex, at least, dilatation of physiological significance occurs just as it does on the brain surface.

## CONCLUSION

Further evidence has been obtained confirming the existence of a parasympathetic vasodilator innervation of cerebral vessels in the parietal region of the cat.

We wish to thank the officers of the Marine Biological Laboratory for generously permitting us to use the facilities of that institution, including laboratory space where this work was carried out. Also we want to thank several members of the M. B. L. physiology class for their enthusiastic help in these experiments.

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# RÔLE OF THE THYROID IN THE CARBOHYDRATE DISTURBANCE WHICH FOLLOWS HYPOPHYSECTOMY[1]

SAMUEL SOSKIN, R. LEVINE AND R. E. HELLER

*From the Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago*

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It is generally recognized that clinical states of hyperthyroidism or hypothyroidism in humans exert a significant effect on carbohydrate metabolism, as judged by hyperglycemia and glycosuria on the one hand, and increased carbohydrate tolerance on the other (1). When thyroid dysfunctions are superimposed on diabetes mellitus, the severity of the diabetes is correspondingly aggravated or diminished (2). Nevertheless a number of attempts to demonstrate this relationship experimentally in animals have led to the conclusion that the thyroid gland has little or no direct influence on carbohydrate metabolism, and that the minor effects which are obtained are due to the increase or decrease in the rate of metabolism (3). More recently Dohan and Lukens (4) have re-investigated the effect of thyroidectomy upon pancreatic diabetes in the cat. The small (though significant) influence which they observed, as compared to the marked effects of hypophysectomy, led them to conclude that the secondary atrophy of the thyroid gland plays a relatively small part in the profound modification of diabetes which follows removal of the hypophysis from the depancreatized animal.

In reviewing the above we were struck by the fact that the various workers almost uniformly neglected to verify the hypothyroid status of their thyroidectomized animals. This seemed particularly important because cats and dogs were used, and because Marine (5) found that aberrant thyroid tissue may be demonstrated in over 90 per cent of these animals. It seemed likely, therefore, that many of the negative and inconsistent results might have been due to the fact that the thyroidectomized animals were not functionally hypothyroid. Since, in our hands, thyroidectomy in adult dogs has failed in most instances to produce signs and symptoms of hypothyroidism, we chose to approach the problem by administering thyroid hormone to hypophysectomized dogs, to compensate for the decreased activity of their thyroid glands.

METHODS. Adult dogs were hypophysectomized by a method slightly

[1] Aided by the Max Pam Fund for Metabolic Research.

modified from that of Dandy and Reichert (6). After complete recovery from the surgical interference when the wounds were well healed, the animals were subjected to the test of fasting. Only those dogs which responded with a prompt hypoglycemia were used (7, 8). The completeness of the hypophysectomy was further verified by post-mortem examination, in all the animals which were sacrificed.

Thyroxine[2] injected intramuscularly, rather than thyroid extract by mouth, was used to obviate the influence of the altered intestinal absorption of these animals on our results. The dosage throughout these experiments was $\frac{1}{2}$ mgm. per kilogram of body weight per day.

Blood sugar was estimated by the Somogyi modification of the Shaffer-Hartman method, and urine nitrogen by the macro-Kjeldahl procedure.

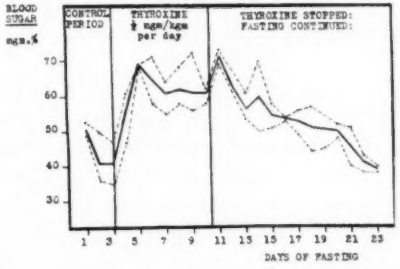



Fig. 1. Maintenance by thyroxine of a normal blood sugar level in a fasting hypophysectomized dog. The upper and lower broken lines, respectively indicate the maximum and minimum blood sugar levels for each day. The heavy continuous line indicates the mean value for all the blood sugar estimations (at least 3 per day) made on each day.

I. *Maintenance by thyroxine of a normal blood sugar level in the fasting hypophysectomized dog.* Five hypophysectomized dogs were subjected to successive periods of fasting with and without the administration of thyroxine. The experiments were varied as to the sequence of the control and thyroxine periods, as to the length of the thyroxine periods, and as regards the insertion of feeding days between the fasting periods. However, all the results were so uniformly positive in demonstrating the effect of thyroxine that, in order to conserve space, figure 1 will suffice as typical of all. It may be seen that thyroxine restores the blood sugar level of the hypoglycemic fasting hypophysectomized animal. Moreover, the continued administration of thyroxine maintains the blood sugar indefinitely, not merely at levels which usually occur in the fed hypophysectomized

[2] We are indebted to Roche-Organon, Inc., through the courtesy of Doctor Shaner, for generous supplies of their synthetic thyroxine.

dog, but at levels which are characteristic of the normal animal. The effects of thyroxine persist for 2 weeks or longer after the last dose has been given. All in all, the thyroxine-treated hypophysectomized dog represented in figure 1 was able to maintain his blood sugar above the hypoglycemic level for 3 weeks, and might have done so for a longer period had thyroxine been continued. This is about as long as some normal dogs can be fasted without showing some degree of terminal hypoglycemia. When one compares the rapidity of the fall in the blood sugar, which occurs within 24 hours in the control period, with the length of the thyroxine and post-thyroxine periods, it is evident that these results cannot be explained by any incidental effect of thyroxine, such as an increased glycogenolysis of preformed glycogen stores. From these data and from our results on other animals, there can be no question but that the ability of the normal animal to maintain a normal blood sugar level under conditions of feeding and fasting, can be restored to the hypophysectomized dog by the administration of thyroxine.

*Comment.* These results indicate that the secondary atrophy of the thyroid gland probably plays an important part in the carbohydrate disturbance which follows hypophysectomy of the normal or depancreatized animal. They also help to clarify the nature of this disturbance. On a priori grounds the hypoglycemic effect of fasting in the hypophysectomized animals must be attributed to a disproportion between the rates of formation and utilization of sugar, due to either or both of the following:

1. An increased rate of utilization of the blood sugar.
2. A decreased rate of formation of blood sugar.

The previous work of Biasotti and Houssay (7) and of this laboratory (8) has favored the latter alternative. More recently, however, Russell and co-workers (9) have concluded that the hypoglycemia is due to an increased rate of utilization of sugar. This conclusion was based chiefly upon the more rapid disappearance of carbohydrate from the tissues of fasted hypophysectomized rats as compared to fasted normal rats. It is obvious, however, that their observation does not warrant their conclusion. The greater loss of carbohydrate in their hypophysectomized animals might just as well have been due to a decreased formation of carbohydrate during the fasting period, with no increase in the rate of utilization. As a matter of fact, we have since published work of a direct nature showing that sugar utilization is markedly diminished in hypophysectomized dogs (10). This indicated that both utilization and formation of carbohydrate were decreased after hypophysectomy, but that the rate of formation was depressed to a greater degree than the rate of utilization. This conclusion is fully supported by the present work, for there is evidence that thyroxine probably increases carbohydrate utilization (11, 12, 13). Its effects in alleviating the hypoglycemia of the hypophysec-

tomized animal must therefore be due to a relatively greater increase in the rate of formation than in the rate of utilization of blood sugar. This is incompatible with the belief that the hypoglycemia is due to an increased rate of utilization of sugar.

II. *Lack of influence of thyroxine on the insulin sensitivity of the hypophysectomized dog.* Insulin was administered to 3 hypophysectomized dogs before and after a 2 day period of thyroxine treatment. The experiments were performed in the post-absorptive state, before the first morning

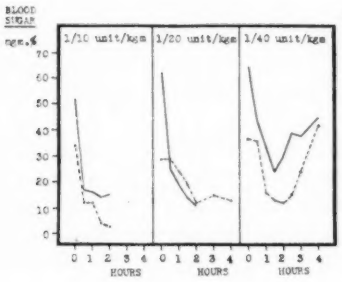



Fig. 2

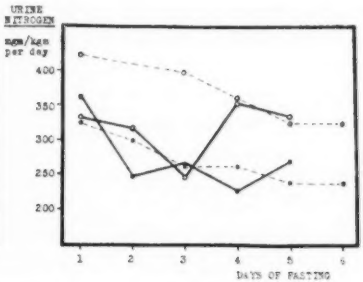



Fig. 3

Fig. 2. Lack of influence of thyroxine on the hypersensitivity to insulin of hypophysectomized dogs. Each set of curves represents a different hypophysectomized animal. In each case the broken line represents the effect of insulin before thyroxine treatment, while the continuous line represents the effect of the same dose of insulin during thyroxine administration. Note the higher initial blood sugar values in the thyroxine treated animals.

Fig. 3. Influence of thyroxine on the total urinary nitrogen excretion of hypophysectomized dogs. The broken lines are taken from the figure published by Houssay (18) representing the results of Braier on fasting hypophysectomized dogs (solid squares) as compared to fasting normal dogs (hollow circles). The heavy continuous lines represent our own results on fasting hypophysectomized dogs (solid squares) as compared to thyroxine-treated fasting hypophysectomized dogs (hollow circles). Note that our results on fasting hypophysectomized dogs are similar to those of Braier on similar animals. After the first 3 days of fasting the results in our thyroxine-treated hypophysectomized dogs are similar to those in Braier's fasting normal dogs.

feeding. The insulin was given subcutaneously in doses which varied in different animals, from 1/40 to 1/10 of a unit per kilogram of body weight. Figure 2 clearly shows that thyroxine does not reduce the marked sensitivity of these animals to insulin. On the other hand, the somewhat greater insulin sensitivity seen in some of the thyroxine treated animals may have been due to decreased stores of liver glycogen (14), the higher initial blood sugar level (15), or a more rapid absorption of the injected insulin.

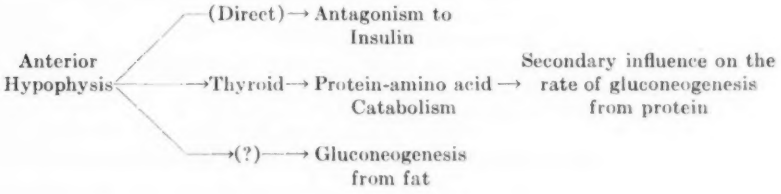
*Comment.* Our results in respect to insulin sensitivity confirm those of Cope and Marks (16). When considered in conjunction with our results on the hypoglycemia of fasting, they indicate that while the secondary atrophy of the thyroid gland is an important factor in the carbohydrate disturbance which follows hypophysectomy, it is not the only factor. Since the adrenalectomized animal exhibits a similar hypersensitivity to insulin, it might be thought that this phenomenon is consequent to the secondary atrophy of the adrenal cortex after hypophysectomy. However, as Houssay (17) has pointed out, anterior pituitary extracts retain their diabetogenic properties and increase the resistance to insulin in the completely adrenalectomized animal. It therefore seems likely that the insulin sensitivity which results from hypophysectomy is due to the lack of a hypophyseal hormone which acts directly on the tissues, and is not mediated by another gland.

III. *Influence of thyroxine on the total nitrogen excretion of the hypophysectomized dog.* In 2 of the fasting hypophysectomized dogs in which the ability of thyroxine to maintain a normal blood sugar level was demonstrated, the urinary nitrogen excretion was determined before and during the thyroxine treatment. The results are given in figure 3, in which they are compared to figures previously reported by Braier (18) for the fasting hypophysectomized and the fasting normal dog. The nitrogen excretion of our fasting hypophysectomized dogs closely approximated that of Braier's similar animals. After the first few days of fasting, the nitrogen excretion of our thyroxine-treated hypophysectomized animals approached that of his fasted normal dogs. It seems probable that the greater nitrogen excretion seen in the normal dogs during the first few days of fasting, may be accounted for by the superior initial nutritional state of these animals as compared to our hypophysectomized dogs, which had already undergone the control fasting period before thyroxine was administered. It may therefore be concluded that thyroxine largely restores a normal rate of endogenous protein catabolism to the hypophysectomized dog.

*Comment.* These results indicate that the secondary atrophy of the thyroid gland is probably responsible for the decreased endogenous protein breakdown which follows hypophysectomy. The decreased rate of formation of sugar in the hypophysectomized animal can be related, in part, to this defect in protein catabolism. The difficulty, however, is not in the gluconeogenetic process itself, for it has been amply shown that the hypophysectomized animal forms sugar very readily from ingested protein (7, 8). When one remembers that exogenous protein is absorbed in the form of amino acids, it seems logical to conclude that the disability in endogenous protein catabolism concerns chiefly the breakdown of tissue protein to amino acids.

Discussion. We have previously concluded (8) that the hypophysec-

tomized animal differs from the normal in that the latter derives sugar from both protein and fat, while the former is unable to convert fat. These considerations in conjunction with our present results, suggest the following tentative scheme representing the various influences of the hypophysis on carbohydrate metabolism.


(Direct) → Antagonism to Insulin
Anterior Hypophysis
→ Thyroid → Protein-amino acid Catabolism → Secondary influence on the rate of gluconeogenesis from protein
→ (?) → Gluconeogenesis from fat

## SUMMARY AND CONCLUSIONS

1. The completeness of thyroidectomy in adult cats and dogs is rendered highly uncertain by aberrant thyroid tissue. This probably accounts for the conclusions, drawn from thyroidectomy experiments, that the thyroid gland has little direct influence on carbohydrate metabolism.

2. The intramuscular injection of thyroxine in hypophysectomized dogs, maintained a normal blood sugar level in them throughout long periods of fasting, increased their urinary nitrogen excretion to that of fasting normal dogs, but did not diminish their hypersensitivity to insulin.

3. The secondary atrophy of the thyroid gland, probably plays an important part in the decreased endogenous protein catabolism and in the related carbohydrate disturbance of the hypophysectomized animal. Thyroid atrophy is apparently not the cause of insulin sensitivity.

4. The nature of the carbohydrate disturbance which results from hypophysectomy is discussed, and a tentative scheme representing the various influences of the anterior hypophysis on carbohydrate metabolism is ventured.

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# DECREASED RESISTANCE TO HYPOGLYCEMIA ON SUCCESSIVE DAYS OF ADMINISTRATION OF INSULIN[1]

WARREN C. CORWIN

*From The Mayo Foundation, Rochester, Minnesota*

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In attempting to determine the effects of various procedures and substances on convulsions caused by insulin in nondiabetic dogs, it was found necessary to develop a standard procedure for purposes of comparison. Such a procedure was devised and strictly adhered to. In each experiment the animal in question was given 1 unit of insulin per kilogram of body weight intravenously every two hours until convulsions occurred. By this method the amount of insulin which was required to produce convulsions and the length of time which elapsed before convulsions occurred could be accurately determined in each case.

The criteria for convulsions were: opisthotonus, clonic spasm of the jaws, labored breathing or actual cessation of respiration owing to closure of the glottis, running movements, loss of sphincter control and salivation. These criteria were demanded for the sake of accuracy in the observations and were rigidly adhered to throughout the study. Though the loss of reflexes is easily demonstrable in dogs and may thus be termed an objective sign, the loss of consciousness is more a subjective symptom than it is an objective sign and it is frequently most difficult if not impossible to determine. Because true convulsions according to the criteria laid down above may occur with or without partial loss of reflexes and because of the difficulty in determining the state of consciousness of the dog, neither the loss of reflexes nor the loss of consciousness was employed as a criterion in this study. Prodromal twitchings, salivation, retching, vomiting, frequency of urination, apathy, muscular weakness and ataxia, amounting at times to an actual inability to stand, were nearly always observed. Approximately 30 per cent of the dogs employed did not have typical convulsions but proceeded directly from their prodromes into a state of coma. Such dogs were considered to be unsuitable for these studies and

[1] A preliminary report of this study appeared in Proc. Staff Meet. Mayo Clinic **12:** 716 (Nov. 10) 1937.

Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D. in Experimental Surgery.

accordingly were not employed. Furthemore, any dog which required more than 4 units of insulin per kilogram of body weight before convulsions occurred was likewise rejected.

The dogs were maintained on constant amounts of a standard mixed diet calculated according to caloric requirements. The diet employed consisted of 38 per cent horse meat, 35 per cent cracker meal, 15 per cent cooked tomatoes, 8 per cent lard and 4 per cent bone ash. An eighteen hour fast preceded each experiment and the experiment was terminated by the intravenous injection of 5 cc. of a 20 per cent solution of *d*-glucose per kilogram of body weight—that is, 1 gram of *d*-glucose per kilogram of body weight.

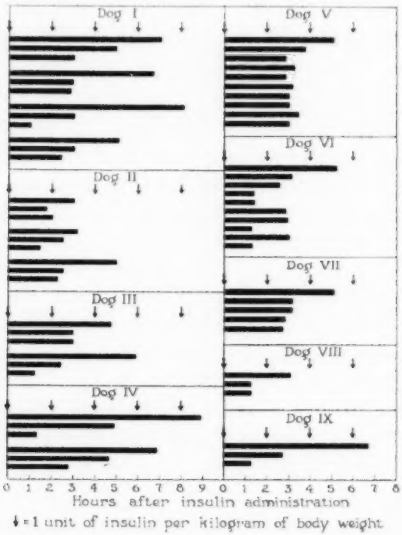
Dog I
Dog II
Dog III
Dog IV
Dog V
Dog VI
Dog VII
Dog VIII
Dog IX
0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8
Hours after insulin administration
↓=1 unit of insulin per kilogram of body weight

Fig. 1. Decreased resistance to hypoglycemia when insulin was administered on successive days. Each line represents the length of time required before convulsions occurred. Dogs 1, 2, 3 and 4 were the subjects of repeated experiments. Dogs 5 and 6 were given insulin on ten successive days.

It was found quite by accident that if a dog is given injections of insulin on successive days, its resistance to that substance is decreased on the second day and is still further decreased on the third day. This decrease in resistance to insulin is manifested by the smaller amount of insulin which is required to produce convulsions and the shorter period of time which elapses before convulsions occur on the second and third days. After the third day, however, the amount of insulin required to produce convulsions becomes more or less stabilized, so that on all succeeding days there is but little variation in the animal's response. In more than 110

experiments on twenty-six dogs this peculiar decrease in resistance to insulin was absolutely invariable. Not only did it occur in all animals tested but it was found to be true of each animal repeatedly (fig. 1). The phenomenon was so striking and so constant that the desirability of investigating it was soon apparent.

The most obvious starting point seemed to be the determination of the blood sugar level during each day's experiment at sufficiently frequent intervals to permit of comparative analysis. A large amount of such data was accumulated but no constant differences could be made out between the blood sugar curves of the three successive days of any particular experiment. Frequently, to be sure, the initial blood sugar values of the second and third days were lower than the initial value of the first day but almost as frequently they were the same or even higher. Again it was found that the level of the sugar on the first day frequently went lower than it did on the second and third days and yet convulsions did not occur. Further, generally speaking there was no difference in the rate of development of hypoglycemia from day to day. This was determined by quarter-hourly blood samples. In contrast to what might be expected the level of the blood sugar often dropped more rapidly on the first day than on succeeding days.

As has been stated, each experiment which was carried out to determine the response of an animal to injections of insulin was terminated by administering to the animal 5 cc. per kilogram of body weight of a 20 per cent solution of *d*-glucose intravenously. Since, in the three day experiments, the dogs had not had this *d*-glucose prior to the first day, it was felt that the *d*-glucose by increasing the production of endogenous insulin or by some other mechanism might have caused the lessened requirements of exogenous insulin on the succeeding day. Accordingly six dogs were given *d*-glucose in the amounts stated above on the day prior to the inauguration of a standard three day experiment. The responses to insulin on the three days were similar in all respects to those noted on all previous occasions. There was in every instance an increased susceptibility to insulin on the second and third days as compared with that on the initial day.

All other controllable conditions were apparently identical on each succeeding day. It was thought advisable, therefore, to investigate certain phases of the physiologic status of the dog after two days of insulin administration and to compare the results thus obtained with those obtained under standard conditions. The questions of 1, the *d*-glucose tolerance; 2, the glycogen content of the liver; 3, the inorganic phosphate values of the serum, and 4, the potassium content of the serum were investigated as likely indicators of the state of affairs, particularly as regards the animal's carbohydrate metabolism.

*Tolerance for d-glucose.* Normal tolerance curves for *d*-glucose were established on six dogs according to the method generally employed in this laboratory. After a twenty-four hour fast blood was withdrawn for determination of *d*-glucose. One-half of a gram of *d*-glucose per kilogram of body weight was then injected intravenously, usually in the form of a 20 per cent solution, following which blood samples were taken at the following time intervals: five, fifteen, thirty and forty-five minutes and one, one and a half and two hours. The normal tolerance curves thus obtained were taken to represent the animal's response to *d*-glucose on the morning of the first day of an experiment which entailed the injection

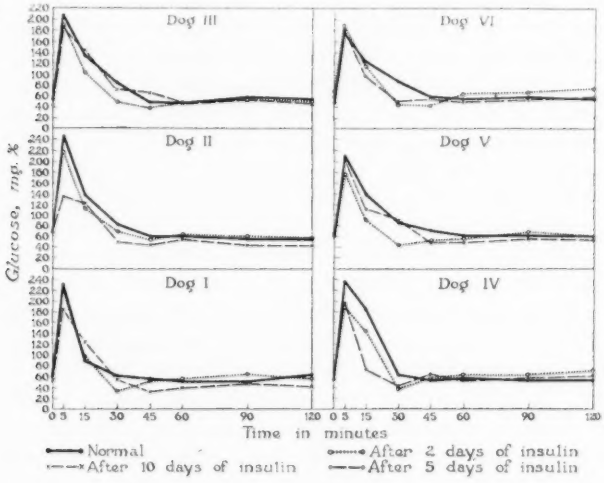


**Fig. 2.** The tolerance curves for *d*-glucose under standard conditions and following successive days of insulin administration.

of insulin on successive days. Tolerance curves for *d*-glucose were next obtained on these animals on the morning following two successive days of experiments to determine the response to insulin. On other occasions tolerance curves for *d*-glucose were established on three of the six dogs after five days of insulin and on the remaining three dogs after ten days of insulin.

A careful appraisal of the data secured (fig. 2) indicates that there is no constant difference in the glucose tolerance as determined in these experiments on successive days of insulin administration.

*The glycogen content of the liver.* Five dogs were chosen for determination of the glycogen content of the liver 1, under standard conditions, and

2, following two days of insulin administration. Determinations were made on tissue removed for biopsy. The quantitative determination of glycogen was made in duplicate employing a modified method of Pflüger. The initial glycogen values of two of the animals were considerably below the normal value and decreased slightly following the administration of insulin. The glycogen content of the other animals remained within normal limits throughout the period of observation (table 1).

*Inorganic phosphate curves.* In order to determine whether decreased resistance to hypoglycemia on successive days of insulin administration might be related to or even dependent on the inorganic phosphate content of the blood, this constituent of the serum was studied during the production of convulsions. The inorganic phosphates of the serum were determined on three dogs at hourly intervals during standard experiments on the response to insulin on three successive days. In these experiments the individual convulsions were typical as was the decreased resistance

TABLE 1

*Percentages of glycogen in the livers of dogs: (A) under normal conditions, (B) following two days of insulin injections*

| | DOG 1 | DOG 2 | DOG 3 | DOG 4 | DOG 5 |
|---|---|---|---|---|---|
| A..... | 3.52* | 2.98 | 4.68 | 5.88 | 7.08 |
| | 3.21 | 3.30 | 5.05 | 5.56 | 7.31 |
| B..... | 2.27 | 1.85 | 5.28 | 4.93 | 7.53 |
| | 2.61 | 2.31 | 4.78 | 5.44 | 7.38 |

* All determinations were made in duplicate.

to insulin noted for each dog on the second and third days. In each instance and on each day of the three day experiments the inorganic phosphates fell (table 2). This is in accordance with the effect of insulin on the blood phosphate first noted by Wigglesworth and co-workers and by Staub, Günther and Fröhlich and since confirmed by Harrop and Benedict and by Briggs and co-workers. A difference was not noted in this response from day to day; that is, the initial value for the inorganic phosphate of the serum and the degree of hypophosphatemia which subsequently developed were neither higher nor lower on the first day than on succeeding days.

*Serum potassium curves.* Since serum potassium is known to decrease after the administration of insulin in a manner quite similar to the behavior of the inorganic phosphate of the serum (1, 3), this element was also investigated during the production of convulsions on successive days of insulin administration. In this series of experiments the study was conducted according to the standard procedure with the single exception that

TABLE 2

*Values for inorganic phosphates of the serum on successive days of insulin administration*

| TIME | DOG 1 | | | DOG 2 | | | DOG 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 |
| *hours* | | | | | | | | | |
| 0 | 4.5 | 4.4 | 4.73 | 4.82 | 4.37 | 4.73 | 5.08 | 5.62 | 5.30 |
| 1 | 3.07 | 2.78 | 2.78 | 2.28 | 2.38 | 2.52 | 3.21 | 2.84 | 3.35 |
| 2 | 2.51 | 2.28 | 2.97 | 2.16 | 2.24 | | 2.98 | 2.56 | |
| 3 | 2.25 | | | 2.20 | 2.13 | | 2.41 | 2.21 | |
| 4 | 2.32 | | | 1.45 | | | 2.75 | | |
| 5 | 2.61 | | | 2.28 | | | | | |
| 6 | 2.30 | | | 2.56 | | | | | |
| 7 | 2.41 | | | | | | | | |

TABLE 3

| DOG | HOURS AFTER INSULIN ADMINISTRATION | SERUM POTASSIUM | BLOOD *d*-GLUCOSE | REMARKS |
|---|---|---|---|---|
| A. Serum potassium and blood *d*-glucose values on the first day of insulin administration (2 units per kilogram of body weight every two hours) | | | | |
| 1 | 0 | 20.6 | 61.7 | Before insulin administration |
| | 0:50 | 16.4 | 33.2 | Muscular twitchings |
| | 3:25 | 13.9 | 28.7 | Semicomatose |
| | 3:35 | 15.2 | 55.5 | Immediately following convulsions |
| 2 | 0 | 21.3 | 58.1 | Before insulin administration |
| | 1 | 12.4 | 22.8 | Semicomatose; twitching |
| | 3:45 | 18.4 | 28.5 | During convulsions |
| 3 | 0 | 21.4 | 65.8 | Before insulin administration |
| | 1 | 14.0 | 34.9 | Apathetic |
| | 4:35 | 14.1 | 28.6 | Retching, vomiting, twitching |
| | 5:45 | 13.4 | 38.5 | Flaccid, semicomatose |
| | 6:15 | 14.0 | 20.0 | During convulsions |
| B. Serum potassium and blood *d*-glucose values on the third day of insulin administration | | | | |
| 1 | 0 | 22.2 | 62.2 | Before insulin administration |
| | 0:30 | 17.0 | 47.2 | During convulsions |
| 2 | 0 | 19.7 | 52.6 | Before insulin administration |
| | 1 | 14.0 | 23.3 | Apathetic |
| | 1:30 | 17.3 | 23.8 | Flaccid, muscular twitching |
| | 2 | 14.3 | 15.1 | Semicomatose |
| | 2:25 | 15.4 | 20.0 | Five minutes after convulsions |

on each day the three dogs employed were given 2 units of insulin per kilogram of body weight every two hours until convulsions occurred. These were all dogs which in preliminary experiments had been found to be somewhat more resistant to insulin than the average dog and which had been rejected from the earlier experiments because of the long time factor involved when only 1 unit of insulin per kilogram of body weight was administered. One of these three dogs died during the night following the first day of insulin administration. It seems likely that its death was caused by overdosage with insulin and the subsequent administration of *d*-glucose sufficient to effect only temporary relief from hypoglycemia.

The serum potassium was determined 1, before the administration of insulin; 2, one hour after the administration of insulin; 3, when possible, at the time when the animal appeared to be the most profoundly shocked but before convulsions had occurred, and 4, either during or immediately after convulsions. This was done on the first and the third days of insulin administration. The decrease in the potassium content of the blood serum on the third day as well as the first day corresponds well with the decreases found by other workers after the injection of insulin (table 3).

## SUMMARY AND CONCLUSIONS

When a nondiabetic dog is given injections of insulin on succeeding days (1 unit per kgm. of body weight every two hours until convulsions occur), convulsions occur earlier on the second day and still earlier on the third day. The resistance to insulin then becomes more or less stabilized so that on all succeeding days there is little if any variation in the response. In our experience of more than 110 experiments on twenty-six dogs this peculiar decrease in resistance has occurred invariably.

No clear-cut reason for this phenomenon has as yet been established. The curve of the blood sugar on the first day does not differ in any way from that on succeeding days. In other words, the levels of blood sugar in fasting dogs, the rapidity of the development of hypoglycemia and the depth of hypoglycemia are similar on all the days of any given experiment. Likewise the phenomenon cannot be explained on the basis of changes in tolerance to *d*-glucose, glycogen content of the liver, inorganic phosphate or potassium of the serum.

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# INTRAMYOCARDIAL PRESSURE AND ITS RELATION TO AORTIC BLOOD PRESSURE

J. RAYMOND JOHNSON AND JOSEPH R. DI PALMA

*From the Department of Physiology, Long Island College of Medicine, Brooklyn, New York*

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That the intramyocardial pressure developed during systole must have some effect on the coronary blood flow has been admitted by most investigators of the circulation. While no direct measurements have been made of the height to which this pressure may rise, many ingenious methods have been developed for determining its effect upon the flow of blood in the coronary arteries. The numerous attempts to quantitate this effect, however, have resulted only in diverse conclusions. Anrep and his associates (1, 2, 3, 4) have repeatedly pointed out in intact animals as well as in heart-lung preparations, and in arteries remaining in connection with the aorta as well as those artificially perfused, that the flow is practically stopped during systole. Their conclusions are based upon hot-wire measurements of inflow into the left coronary artery and upon pressure determinations from the artery peripheral to a point which is clamped during various phases of the heart's cycle. In later experiments Anrep, Davis and Volhard (3) found that there was a forward flow during systole as well as during diastole, which they attributed to the expansion of the large elastic coronary branches rather than to any intramural flow.

Wiggers and his associates (5, 6, 7), on the other hand, believe that there is a significant flow of blood through the intramural vessels of the left ventricle during systole as well as diastole. In a series of investigations, using both artificially and naturally perfused coronary arteries, they found that the pressure in the coronary artery which has been momentarily shut off from the aorta or perfusion reservoir falls even during systole until it reaches a level considerably below that existing in the aorta. The recent work by Green, Gregg and Wiggers (7), in which they repeated the clamping procedure which Anrep described (4), seems to have established definitely that the peripheral holding pressure in the left coronary artery during systole is well below aortic pressure. From these experiments they conclude that "the pressure exerted upon the intramural vessels is definitely less than that created in the left ventricular cavity."

That these two groups of investigators should, after several years of

investigation by practically the same methods, arrive at such diverse conclusions, would seem to indicate the need of some direct method for measuring the intramyocardial pressure. The importance of this factor in the interpretation of flow measurements has been recently emphasized by Katz (8) but only qualitative relations have been considered. The present investigation has been directed toward finding a method whereby the quantitative relations between intramyocardial and aortic pressures can be determined.

METHOD. Our investigation started with the attempt to use the occluded coronary sinus for measuring the pressure of the fluid forced into it by the contracting heart muscle. We soon learned, however, as did Gregg (9), that the maximal pressure attainable in such a system seldom rises as high as aortic or left intraventricular pressure. This may possibly be accounted for by the fact that there are many inaccessible channels through which blood may leave the coronary sinus. It then occurred to us that it might not be impossible to produce an artificial cavity within the heart wall from which pressures could be measured, and with this thought in mind we discovered that a section of resected carotid artery could be sewed into the myocardium with comparative ease. The following experiments were performed on animals prepared in such a manner.

Cats weighing from 3 to 4 kgm. and anesthetized with dial urethane (Ciba) were used. Under artificial respiration the chest was opened by a midline incision and the heart suspended in a cradle made by stitching the pericardium to the chest wall. With the aid of a round, non-cutting needle and silk thread a section of isolated carotid artery about an inch and a half long, obtained usually from the same animal, was passed through the heart wall (see fig. 1). The location chosen varied somewhat in different hearts depending upon the position of visible coronary vessels, but usually it was to the left of the ramus descendans anterior and in a direction parallel to it. About one-half inch of the artery was imbedded in the muscle although the exact length we found made no difference. The size of the artery used was also not important (provided it was not excessively large) as was shown by comparing the results obtained from a section of internal mammary artery with those from the carotid. The depth at which the artery was imbedded in the heart wall, however, was found to be a very important factor as we shall indicate later.

A diagram of the set-up is shown in figure 1. The carotid section was cannulated at both ends, one leading to an optical manometer and the other to the pressure line supplying the manometer. By opening stopcocks *c*, *d*, *e*, and *f* the entire system could thus be filled to any desired pressure and by closing *c* and *f* the pressure changes occurring in the imbedded carotid section could be recorded. This method, which we

shall refer to as method II in subsequent discussions, provides for a back flow into the length of rubber tubing and the mercury manometer between *e* and *f*. It is quite obvious that under these conditions the pressure changes transferred to the optical manometer will be very slight as long as the section of artery remains open. If, however, the artery is completely occluded at any point, the pressure changes between this point and the manometer will be shown by the record. Now if the carotid section be ligated at the point marked by the asterisk, a different system of recording is instituted whereby fluid can be trapped at any desired

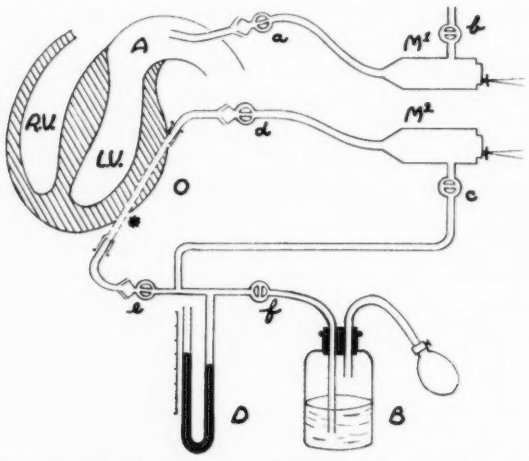


Fig. 1. Diagram showing artery imbedded in the heart wall with its connections to the optical manometer and pressure line.

*A*, aorta; *B*, pressure bottle; *O*, carotid artery imbedded in the myocardium; *D*, mercury manometer; *L.V.*, left ventricle; $M^1$, $M^2$, optical manometers; *a*, *b*, *c*, *d*, *e*, *f*, stopcocks.

The asterisk marks the point of ligation of the carotid section (O) as used in method I. Discussion in text.

pressure in a closed system within the heart wall. This latter system we will designate as method I. A second manometer which connected to the aorta for recording aortic blood pressure completed the set-up for any one experiment.

Due to the very small volume of fluid present in the imbedded portion of artery, very little movement of fluid could take place and it was extremely important that the elasticity of the recording system be kept at a minimum. Since the only elastic part of the system aside from the rubber membrane on the optical manometer was the artery itself, it was necessary that certain precautions be taken in adjusting the cannula which

connected the manometer with the artery. If it were placed too close to the heart the myocardium bumped against the open end of the cannula and occluded it, and if it were placed too far away the efficiency of the recording system was markedly cut down due to the elasticity of the arterial wall.

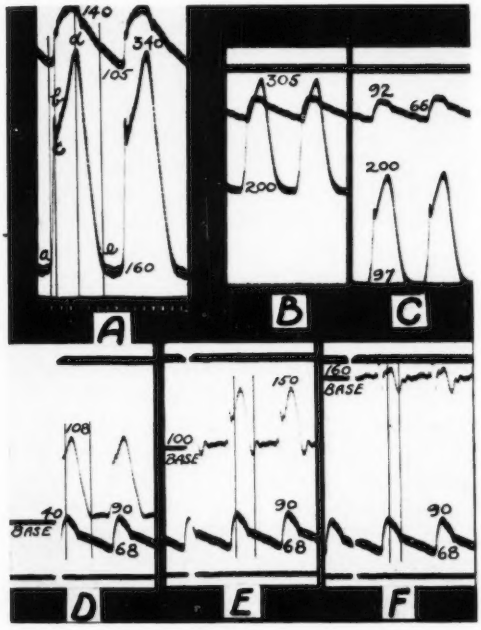


Fig. 2

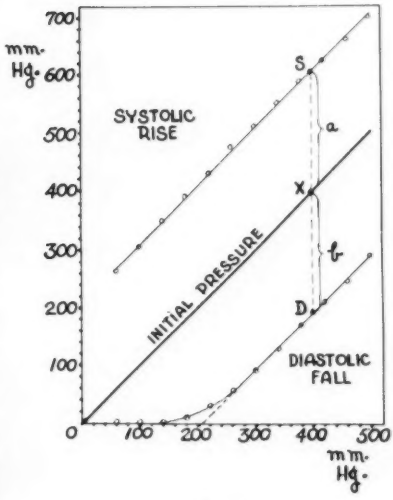


Fig. 3

Fig. 2. A, Contour of the intramyocardial pressure curve and its relation to aortic pressure.

B, C, Intramyocardial pressure curves (method I) showing the systolic rise and diastolic fall from an initial distending pressure of 200 mm. Hg.

D, E, F, Records illustrating method II for determining the maximal intramyocardial pressure. Time in twenty-fifths of a second. Descriptions in text.

Fig 3. Chart showing the constancy of the systolic rise and diastolic fall (method I) over a range of from 60 to 500 mm. Hg initial distending pressure. Ordinate values show the rise or fall of pressure, while the distending pressure is represented on the abcissa.

$X$ = initial distending pressure, $a$ = systolic rise, $b$ = diastolic fall, $S$ = systolic maximum, $D$ = diastolic minimum. Description in text.

*The contour of the intramyocardial curve.* Method I (i.e., with the peripheral end of the carotid section ligated) should give us a curve which represents the contour of the pressure changes occurring in the heart wall. Such a curve is shown in figure 2A (lower curve) which is

typical of the results we obtained. A study of the time relations between this and the accompanying aortic curve reveals the following characteristics of the curve: For about 0.03 second before the primary steep rise of the aortic curve (i.e., before the opening of the aortic valves) the intramyocardial pressure rises brusquely (*a*–*b*). Point *b* on the curve is coincident with the opening of the valves and from here the curve falls sharply to *c*. The further rise beginning at *c* is still steep, although not as abrupt as from *a* to *b*, and comes to a termination at *d*, a point which coincides with the end of systole on the aortic curve. From the very beginning of diastole the curve starts to decline, somewhat less rapidly at first but steeply during the isometric relaxation phase (*d*–*e*). By mid-diastole the relaxation is complete. As one might expect, the time relations of the curve follow closely those of an intraventricular pressure curve. The contour, however, is markedly different. The summit is more irregular and rises to a much sharper peak, and the diastolic decline in pressure is more prolonged, continuing through a large part of diastole.

Our analysis of the intramyocardial pressure curve compares favorably with that obtained by Gregg et al. (6) who cannulated the peripheral end of the anterior descendans coronary ramus. Their curve, however, fails to demonstrate the transitory drop in pressure coincident with the opening of the semilunar valves. Since this was a constant finding in our experiments, and since its magnitude depended upon the distending pressure within the artery, we feel that the drop in pressure is not an artifact but rather that it indicates a transitory release of tension in the heart muscle due probably to the facilitation of blood flow as the valves open. At low distending pressures, comparable to those which Gregg used, we also obtained curves which failed to show this feature.

*The means of obtaining the systolic maximum intramyocardial pressure.* In order to interpret our results an analysis of the physics of our set-up is necessary. The artery section must be thought of as an easily collapsible tube, yet one which, when exposed to an adequate internal pressure, becomes sufficiently rigid to obviate any fluid displacement with further pressure changes. This condition is fulfilled by the artery section only when the internal pressure reaches 200 mm. Hg or above, for it is in this region that its volume-elasticity coefficient approaches infinity. In such a system any pressure increment applied to the outside surface will produce a constant and equal increment of pressure on the inside. Experiments verifying this were carried out on an artificial artery constructed out of gooch crucible rubber tubing enclosed in a jacket of non-distensible cloth and upon an isolated section of carotid artery such as we used.

Applying this principle to the heart, it should be possible to determine the pressure developed by the myocardium, as it contracts against the imbedded artery section, by measuring the rise in pressure within the

artery. That this rise is constant for a given heart, as long as the initial distending pressure remains above 200 mm. Hg, is shown by the results which we obtained. Several experiments were run in which the systolic rise and the diastolic fall for a particular heart were determined at 40 mm. steps from 0 to 500 mm. Hg distending pressure. The data from one such experiment was compiled into the graph shown in figure 3. A moment's consideration will make clear how this was done. First the desired distending pressure was introduced into the whole system with the aid of the pressure bottle. Then, by closing stopcock *c* (fig. 1) at a particular moment, fluid could be trapped at this pressure in the artery section either during diastole or during systole. When it was closed during diastole the contraction of the myocardium during the next systole exerted a force upon the fluid which raised the pressure, hence the systolic rise. A record of this is included in figure 2B. Fluid was trapped at 200 mm. during diastole and rose to 305 mm. during the subsequent systole. In a similar manner, when fluid was trapped in the artery section during systole, the pressure in the recording system fell during the next diastole. The record in figure 2C shows that when the initial pressure was at 200 mm. during systole it fell to 97 mm. during the subsequent diastole. In this manner the data were obtained for the graph (fig. 3), a simple analysis of which shows: 1, with any initial distending pressure ($x$) above 60 mm. Hg the systolic rise ($a$) is always the same; 2, when the initial distending pressure is increased to 260 mm. Hg the diastolic fall ($b$) also becomes a constant quantity; 3, within these ranges of pressure the systolic rise and the diastolic fall are equal, and in this experiment amount to 200 mm. Hg. This, according to our analysis, represents the rise in intramyocardial pressure during systole.

As a further check upon the method we altered the set-up in such a way that a run-off from the artery was provided (method II). This was accomplished by connecting the free end of the carotid section to the pressure system supplying the manometer. Here the dynamic conditions are totally altered from those in method I. Reference to the diagram in figure 1 will make it clear that in order to cause any appreciable rise of the pressure in the manometer the heart muscle must first occlude the carotid artery section at some point. This we decided would occur at any initial distending pressure less than the intramyocardial pressure developed during systole. However, when the initial distending pressure was the greater, no rise would occur in the pressure in the manometer. Our procedure was to record first a base line of the initial distending pressure with stopcocks *c*, *d*, *e* and *f* all open. Stopcock *c* was then closed, thus permitting registration of the same distending pressure during diastole and in addition any increase in pressure which might be developed if the artery became occluded during systole. This gave us a means

of visualizing whether or not the carotid section had become occluded. Curves D, E and F in figure 2 are selected from one such experiment, showing the intramyocardial curve with initial distending pressures of 40, 100 and 160 mm. Hg respectively, while the aortic pressure (lower record) remained constant at 90/68. The significant portion of the intramyocardial curve is that between the vertical lines, showing a smooth, sustained or rising summit up to the end of systole. The actual rise is not significant and becomes progressively less as the distending pressure is elevated until a level is finally reached at which it no longer occurs.

TABLE 1

| EXPERIMENT | AORTIC PRESSURE | INTRAMYOCARDIAL PRESSURE | DEPTH OF ARTERY* | LOCATION OF ARTERY IN THE HEART WALL |
|---|---|---|---|---|
| | | *mm. Hg* | | |
| 5 | 107/80 | 160 | $\frac{3}{4}$ | Right of ramus descendans anterior |
| 7 | 30/24 | 40 | $\frac{3}{5}$ | Right of ramus descendans anterior |
| 8 | 71/45 | 100 | $\frac{1}{2}$ | Right of ramus descendans anterior |
| 14 | 85/40 | 145 | $\frac{1}{2}$ | Left of ramus descendans anterior |
| 9A | 135/118 | 150 | $\frac{1}{2}$ | Left of ramus descendans anterior |
| 9B | 90/58 | 140 | $\frac{1}{2}$ | Further left than 9A |
| 12A | 92/67 | 110 | $\frac{1}{2}$ | Left of ramus descendans anterior |
| 12B | 70/57 | 60 | Very superficial | Placed superficial to 12A |
| 13A | 143/108 | 180 | $\frac{1}{2}$ | Left of ramus descendans anterior |
| 13B | 110/85 | 65 | Very superficial | Placed superficial to 13A |
| 11A | 122/102 | 220 | $\frac{3}{4}$ | Left of ramus descendans anterior |
| 11B | 120/100 | 120 | Very superficial | Placed superficial to 11A |
| 10A | 100/82 | 180 | $\frac{1}{2}$ | Left of ramus descendans anterior |
| 10B | 80/60 | 170 | $\frac{9}{10}$ | Placed deeper than 10A |
| 6A | 114/87 | 260 | $\frac{3}{4}$ | Left of ramus descendans anterior |
| 6B | 120/88 | 180 | $\frac{1}{2}$ | Placed superficial to 6A |
| 6C | 115/80 | 75 | Very superficial | Placed superficial to 6B |

* Expressed in relation to the thickness of the ventricular wall.

This level would correspond to the systolic intramyocardial pressure and in the experiment referred to above it was about 170 mm. Hg.

Several experiments using both methods on the same heart and under identical dynamic conditions proved them to check closely. Satisfied that either method was reliable, we compiled the data in table 1 from 10 experiments using both methods.

*Intramyocardial pressure at different depths of the heart wall.* We usually found it most convenient to run our carotid section through the wall on the anterior surface of the heart and parallel to the ramus descendans anterior. We kept away from the apex, however, feeling that the twisting motion of the fibers in that region might give us unreliable readings. A fact that we had anticipated was confirmed early in the investigation,

viz., that intramyocardial pressure varies with the depth at which the carotid section is placed in the heart wall. Reference to table 1 makes this clear. Five experiments were performed in which the intramyocardial pressure was obtained at different depths. For example, experiment 11A shows that at a depth of three-fourths the thickness of the ventricular wall the pressure was 220 mm. Hg; at a very superficial depth (one-tenth the thickness of the wall) it was only 120 mm. Hg. The aortic pressure remained constant throughout. Experiment 6 shows the pressure at three different depths. No other conclusion can be drawn than that the intramyocardial pressure diminishes gradually from the deeper to the more superficial layers.

*The magnitude of intramyocardial pressure as compared with aortic pressure.* In all of our experiments there was not a single instance in which the intramyocardial pressure at the end of systole did not exceed systolic aortic pressure when the carotid section was placed at a depth of one-half the thickness of the ventricular wall. That this holds true over a wide range of aortic pressures is seen by reference to the table. An attempt to find a proportional relation between the two, however, was fruitless, for intramyocardial pressure seems to depend for its systolic maximum upon the particular quality of heart involved. In the more superficial layers of the heart it may be less than aortic pressure as in experiments 12, 13 and 6. Where a strongly contracting heart is concerned, on the other hand, the pressure even in the more superficial layers may exceed aortic pressure as in experiment 11.

Discussion. After careful reflection we can see no reason why the effect of the myocardium on an isolated vessel inserted artificially into the heart wall should be essentially different than it is upon a naturally imbedded one. It is inconceivable that either can be protected from the action of the heart muscle during its contraction. On the other hand our set-up has certain advantages in that no neuro-vascular reactions can be involved and the blood supply to the heart muscle is not interfered with as it is when a coronary artery is cannulated. Careful observation during the course of our experiments demonstrated that the sewing of the carotid section into the myocardium had little if any deleterious effect. Seldom was there any appreciable bleeding and never was there any gross evidence of infarcted areas.

That the possible stretching of adjacent muscle fibers by the distended vessel could not have been a factor of any importance is borne out by the identical results obtained when the carotid was replaced by the internal mammary, a vessel of much smaller size. The parallel course of the initial pressure and the systolic maximum in figure 3 also indicates that there could have been no effect of stretching beyond 60 mm. Hg diastolic pressure.

We regard these results in the nature of a supplement to the work which Wiggers and his associates have done. The idea of a gradient in the intramyocardial pressure is neither original nor new, but our ability to demonstrate that it does exist and to compare it quantitatively at different levels with the aortic pressure has provided a basis for explaining further their results and for correlating them with those of Anrep and his associates. Indeed, both of these groups of investigators almost anticipated our finding. Anrep has repeatedly pointed out that perhaps only in strongly contracting hearts is the flow completely arrested during systole, and his work with Davis and Volhard shows that there may be a definite forward movement of blood during systole as well as during diastole. In this respect it is only his interpretation of such a result which differs markedly from that of Green, Gregg and Wiggers. The latter group, although they point out the fallacy in Anrep's explanation, fail in drawing their own conclusions to take into account a condition which they themselves postulate and which we have been able to supply proof of in this investigation, viz., the gradient of pressure from the internal to the external surface of the ventricular wall. Their differential pressure curves of aortic and peripheral coronary pressure clearly demonstrate that there is a considerable coronary flow even at the peak of systole. It must be pointed out, however, that the peripheral holding pressure determined in this manner is that represented by the portion of the coronary bed where there is least resistance to flow. From our results we are able to arrive at the conclusion that such curves apply only to the more superficial layers of the myocardium, for here alone is there the dynamic possibility of flow. It is therefore possible, when studying the intact coronary bed, to obtain a result of forward movement of coronary blood even at the height of systole although most of the deeper coronary vessels are being completely occluded. In strongly beating hearts, on the other hand, where the intramyocardial pressure in even the more superficial layers may be higher than aortic pressure, there may be complete cessation of blood flow as Anrep has pointed out.

It must not be inferred from this discussion that the blood supply to the deeper layers of the ventricular wall is negligible during systole. While our experiments indicate that there is the dynamic possibility of complete occlusion of blood vessels in the more internal layers of myocardium, this does not mean that the flow stops completely for the whole of systole. It is only at the systolic peak that our results show that occlusion may occur, and thus it is probable that the flow is retarded only momentarily which is not untenable physiologically as some investigators seem to believe.

SUMMARY

A method of optical recording is described for measuring directly the extent of the intramyocardial pressure at any desired depth in the wall of the left ventricle, and the contour of such pressure curves is described. By means of simultaneous records of the pressure from the ventricular wall and from the aorta the following important relations are found to obtain:

1. During the height of systole there exists in the wall of the left ventricle a gradient of pressure decreasing from the deeper to the more superficial layers.

2. In the depth of the myocardium this pressure is always greater than aortic pressure but in the superficial layers it may be equal to or even less than the pressure in the aorta and coronary arteries.

These results indicate that while the deeper coronary vessels must become completely occluded during the height of cardiac contraction there may be a continuous forward movement of blood in the more superficial layers of the myocardium.

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# THYROID STIMULATION BY COLD

## Including the Effect of Changes in Body Temperature upon Basal Metabolism

G. C. RING

*From the Department of Physiology in the Harvard Medical School*

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During short exposure to cold the adrenal medulla may be thrown into action and elevate metabolism (see Cannon, Querido, Britton and Bright, 1927). There is considerable evidence that the thyroid gland also becomes more active if the cold is continued over a long period of time. Mills (1918), using morphological methods, found indications that low external temperatures increase thyroid activity. Riddle (1927) showed that in pigeons the thyroid enlarges in size during the autumn and winter months and decreases during the spring and summer. Seidell and Fenger (1912) discovered that the thyroid glands of sheep, beef and hogs contain about three times as much iodine between the months of June and November as between December and May. Kendall and Simonsen (1928) reported an increase in both the iodine and thyroxin during the summer but a decrease during the winter. On the other hand, Vallesi (1934), using guinea pigs exposed to cold for as long as 40 days, was unable to confirm the correlation between iodine content of the thyroid and external temperature. Hildebrandt (1921) noted no difference in the regulatory metabolism in normal and thyroidectomized animals placed in cold surroundings; and Cori (1922) observed that, after cooling, rabbits without thyroids raised their body temperatures as fast as normal animals. These last two observations mean only that the thyroid gland is not essential for the maintenance of body temperature.

Workers differ as to the duration of exposure to cold that is required to produce *histological* changes in the thyroid. Ludford and Cramer (1928) stated that epilated rats placed in an ice-box for 24 hours manifest marked secretory activity in the cells of the thyroid gland and intense congestion of its capillaries. A prompt increase in blood flow through the thyroid resulting from exposure to cold has been reported by Dietrich and Schwiegk (1932). On the other hand, Kenyon (1933) declared that animals living at a temperature between 0 and −3°C. for 6 to 24 hours show nothing in particular; 10 to 25 days brought about hypertrophy, hyperplasia, loss of colloid and increased vascularity of the gland. Kuschinsky

(1935) believed that during the first 10 days of living at 4°C., the thyroid glands of rats are resting—after 25 days, an active appearance was observed. Woitkewitsch (1935) also reported that 7 days at 2°C. are not long enough to produce microscopic changes in the thyroid of rats but that 25 days do suffice. Wolf and Greep (1937), studying the control of the thyroid gland, showed histological changes similar to those outlined above.

Although there is general agreement that prolonged life in the cold brings about structural changes in the thyroid, this does not prove that increased release of hormone and stimulation of metabolism occur. The only testimony on this point which I have found is that given by Schenk (1922). He recorded that after cooling a normal rabbit the blood serum was so changed that when it was injected into a thyroidectomized animal the recipient's metabolism was stimulated. If the thyroid was extirpated from the donor his serum was no longer affected by lowering the body temperature.

Rats living for one day at 21°C. instead of 28°C. have an elevated basal metabolism (see Benedict and MacLeod, 1929). The thyroid gland, however, is not necessary for the response (see Ring, 1936). In the present paper it will be shown that changes in basal metabolism and body temperature run parallel. Rats do not regulate body temperature well, and so, after they have been exposed to cold, it is not surprising that their temperature usually overshoots the normal level. From results obtained in this way, however, one may calculate a factor which will correct metabolic measurements for changes in body temperature. Thus it has been possible to obtain evidence on the effect of long-continued cold upon thryoid secretion.

Methods. The apparatus was the same as that used earlier (see Ring, 1938). Simultaneous records of basal metabolism and body temperature of rats living in an environmental temperature of 30°C. were obtained. Two groups of experiments were performed; group A, with brief exposure to cold, and group B, with prolonged exposure.

*Group A.* Normal animals in the post-absorptive state were studied first, after they had spent the night in a warm room. Then, a week later, the basal metabolism was measured at the usual bath temperature of 30°C., after the rats had stayed for 4 hours in the metabolism apparatus with the bath temperature lowered to 20°C. In the third and fifth weeks of the experiment the rats were not exposed to cold before measuring the basal metabolism. In the fourth and sixth weeks, 4 hours in the cold preceded the metabolic studies. The results for the first, third and fifth weeks (without previous exposure to cold) were averaged and compared with figures for the second, fourth and sixth weeks. In order to learn the importance of the thyroid gland in these circumstances, completely thyroidectomized rats were studied in the same manner.

*Group B.* In this group of experiments two or more metabolic studies were made first on normal rats. They were then placed in a refrigerator which was maintained at a temperature between 0° and 5°C. A sojourn in the cold for 2 weeks or less did not regularly produce any change in metabolism. Only at the end of 3 weeks were the animals removed from the refrigerator; thereupon they were kept over night at room temperature (25°C.) in preparation for a metabolic study the next day at the environmental temperature of 30°C. This completed, the rats were returned to the refrigerator, and after 7 days measurements were taken to check those of the previous week. From then on, the animals lived at room temperature; their metabolism was determined weekly until it returned to normal. Usually one week was required. In that case the results were checked during the second week to see if the metabolism would become subnormal. It never did.

In order to learn whether the thyroids are important in elevating metabolism during long exposure to cold, these glands were extirpated from a number of rats. After allowing 3 weeks for recovery and making suitable preliminary observations, the rats were placed in the refrigerator. One out of 10 operated rats survived more than 9 days. This animal, whose metabolism indicated incomplete thyroidectomy, lived in the cold for 3 weeks and showed a slight increase in metabolism. Of two thyroidectomized rats, given 1 mgm. each of thyroxin, one died on the 14th and the other on the 18th day of exposure to cold. I finally adopted the plan of removing only about 95 per cent of the thyroid tissue. Out of 15 of these rats placed in the refrigerator 3 survived for the 4 weeks needed to compare them with normal animals.

All rats were fed a diet of "Purina Dog Chow" supplemented weekly with greens. Such a diet contains adequate amounts of iodine to keep the histological appearance of the thyroids normal. In preliminary experiments, animals were given each day a subcutaneous injection containing 10$\gamma$ of iodine but this did not change the metabolic stimulation produced by cold and so was discontinued.

Results. Table 1 presents the results of the group A experiments (brief exposure to cold). The measurements of basal metabolism and body temperature of normal and thyroidectomized rats, with and without previous exposure to cold, have been averaged. Each figure represents at least three determinations made over a period of 5 weeks or more. The largest variation from the mean for observations on each animal is 7 per cent; the probable error for metabolism averages for normal animals without exposure to cold is $\pm 10$ and with previous exposure to cold is $\pm 15$; the figures are about the same for the thyroidectomized animals. Rat 5 is the only one which does not show an elevation of body temperature and metabolism after the exposure. This rat, however, does show the

correlation between temperature and metabolism. The essential relation is this one and not the relation between previous environmental temperature and metabolism. The results on rat 5 have been omitted from the averages. The parallelism between body temperature and metabolism is also suggested when one compares the observations on different animals, as shown in the first two or last two columns of the table.

Examination of the results from thyroidectomized rats shows that their body temperature and metabolism are a little less stable than those of the controls. The relation between these factors is similar in the two groups

TABLE 1

*Group A experiments*

*Basal metabolism and body temperature of normal and thyroidectomized rats with and without previous exposure to cold*

| RAT NUMBER | WITHOUT EXPOSURE TO COLD | | AFTER EXPOSURE TO COLD | |
|---|---|---|---|---|
| | Cal. per sq. m. per day | Body temperature | Cal. per sq. m. per day | Body temperature |
| | | °C. | | °C. |
| Normal: | | | | |
| 1 | 751 | 37.7 | 819 | 38.1 |
| 2 | 769 | 37.7 | 803 | 38.0 |
| 3 | 808 | 38.3 | 869 | 39.1 |
| 5 | (811) | (38.3) | (724) | (37.7) |
| 6 | 837 | 38.3 | 869 | 39.1 |
| Average | 791 | 38.00 | 840 | 38.57 |
| Thyroidectomized: | | | | |
| 7 | 566 | 36.8 | 653 | 37.7 |
| 9 | 571 | 37.1 | 641 | 38.6 |
| 10 | 620 | 38.0 | 705 | 39.2 |
| 11 | 623 | 37.1 | 688 | 38.0 |
| 12 | 639 | 37.6 | 751 | 38.9 |
| Average | 604 | 37.32 | 688 | 38.48 |

All measurements were made at an environmental temperature of 30°C.

and may be expressed by making use of the Arrhenius equation. The $\mu$ value of this equation for the results on normal animals comes out 20,300 and for those on thyroidectomized 21,700. Since these figures are close together, it is probable that the $\mu$ value is unaffected by thyroidectomy. The point should be emphasized, however, that these results are not to be considered extremely accurate because the changes in temperature were small, and also because mean body temperature was not determined but only the temperature of a small part of the colon. It is interesting that the figures obtained agree with observations made by

DuBois (1921) on patients with fever (see also Carpenter, 1933). He showed an average temperature coefficient of 2.3 between 37° and 40°C. I have calculated the $\mu$ value for these figures to be 21,300. It would seem unlikely, therefore, that in the temperature range now studied the value

TABLE 2

*Group B experiments*

*Basal metabolism and body temperature of normal and thyroidectomized rats before and after living in a refrigerator for at least 3 weeks*

| RAT NUMBER | BEFORE LIVING IN REFRIGERATOR | | WITHIN 24 HOURS AFTER LEAVING REFRIGERATOR | | AT LEAST 1 WEEK AFTER LEAVING REFRIGERATOR | | INCREASE IN METABOLISM DUE TO COLD |
|---|---|---|---|---|---|---|---|
| | Cal. per sq. m. per day | Body temperature | Cal. per sq. m. per day | Body temperature | Cal. per sq. m. per day | Body temperature | |
| | | °C. | | °C. | | °C. | per cent |
| Normal: | | | | | | | |
| 13 | 843 | 37.7 | 1004 | 39.0 | 789 | 38.2 | 23 |
| 15 | 835 | 37.7 | 1000 | 38.5 | 839 | 37.8 | 20 |
| 16 | 784 | 37.8 | 938 | 37.6 | 784 | 37.8? | 20 |
| 17 | 795 | 37.1 | 1076 | 39.0 | 847 | 38.2 | 31 |
| 18 | 773 | 37.7 | 852 | 36.9 | 806 | 38.3 | 9 |
| Average | 806 | 37.60 | 974 | 38.20 | 813 | 38.06 | 21 |
| Partially thyroidectomized: | | | | | | | |
| 14 | 770 | 37.9 | 852 | 38.6 | 842 | 38.0 | 5 |
| 20 | 776 | 37.6 | 832 | 36.8 | 821 | 37.2 | 4 |
| 8 | 690 | 37.7 | 818 | 37.2 | 795 | 37.2 | 10 |
| Average | 745 | 37.73 | 834 | 37.53 | 819 | 37.47 | 6 |
| Superior cervical ganglia removed: | | | | | | | |
| 22 | 823 | 37.7 | 1000 | 37.6 | 768 | 37.1 | 26 |
| 4 | 797 | 37.6 | 1006 | 38.6 | 779 | 36.9 | 31 |
| 21 | 811 | 37.7 | 945 | 38.8 | 773 | 38.2 | 19 |
| 23 | 723 | 37.2 | 918 | 38.1 | 718 | 37.0 | 27 |
| 24 | 786 | 37.4 | 1085 | 37.1 | 828 | 37.8 | 34 |
| Average | 788 | 37.52 | 991 | 38.04 | 773 | 37.40 | 27 |

All measurements made at an environmental temperature of 30°C.

should be as low as 16,100—a figure obtained for rats with body temperatures below 33°C. (see Ring, 1938).

Table 2 presents the results of the group B experiments—those in which the rats lived in a refrigerator for 3 weeks and longer. These figures are averages of at least two observations. With one exception, all the nor-

mal animals had a much greater increase in metabolism than did the partially thyroidectomized. Rat 18, after being 3 weeks in the refrigerator, had a metabolism of 764 cal. per day, practically normal, but the body temperature was low, 36.1°C. If the metabolism figure is corrected for the subnormal temperature, this rat shows an increase amounting to about 20 per cent. Furthermore, after the fourth week in the refrigerator, rat 18 had a metabolism of 940 cal. and a body temperature of 37.7°C. The figures in the table are the mean of these two observations. Aside from these, the results which were averaged checked quite closely. Also in all animals which were tested the response to cold occurred a second time. In rat 13 the increase in metabolism was 23 per cent on one occasion and 16 per cent on another when the refrigerator was not kept so cold.

Among the thyroidectomized rats on which there are complete data cold produced a mean increase in basal metabolism of 6 per cent. In calculating this figure, the preliminary and final basal metabolic rates have been averaged. If only the preliminary measurements are considered, the difference is about 10 per cent. It is the same for incomplete observations on two other rats. The final figures reveal no appreciable reduction in metabolism after the return to room temperature, even though observations are continued for a month or more. It is suggested that the thyroid only reaches its normal level of hormone secretion while these animals are living in the refrigerator and therefore the return to room temperature does not reduce thyroid activity. An indication that the thyroid remnant hypertrophies continuously during the refrigerator experience, is provided by the fact that the second metabolic observation during life in the cold is always slightly higher than the first. This is not true for the tests on normal animals.

In order to make sure that changes in body temperature are not interfering with the interpretation of the results, the $\mu$ value, as determined above, may be used for making suitable corrections. We then obtain an average increase in the metabolism for the normal rats of 16 per cent and for the partially thyroidectomized of 7 per cent. The difference seems much too large to be accidental.

The third series of results in the table discloses what happens when the superior cervical ganglia are removed. Even when a temperature correction is included, the increase in metabolism amounts to 18 per cent—similar to the figure for normal rats. This shows that simply operating in the neck does not upset the stimulating effect of cold, provided the thyroid is present. It also shows that whatever the mechanism which brings about the thyroid stimulation, impulses need not pass through the superior cervical ganglia (see Friedgood and Bevin, 1939). This is interesting in view of the observations of Florentin, Fontaine and Hennequin (1936). These authors reported that histological changes took place in the thyroid of rabbits after the superior cervical ganglia were removed.

It is important to point out that lack of light is not the stimulus which elevates metabolism during the stay in the refrigerator. Both Bergfeld (1930) and Turner and Benedict (1932) found that histological changes were produced by the lack of light but that no change in metabolism occurred. In my experiments only when metabolic changes were produced darkness was associated with the application of cold or heat.

## SUMMARY

1. The elevation in the basal metabolism of rats which follows short periods of exposure to cold is associated with a rise in body temperature (see table 1).

2. The $\mu$ value of Arrhenius, determined at normal body temperatures of rats, is about 21,000. It is not affected by thyroidectomy.

3. Living for 3 weeks or more at 0° to 5°C. produced in rats an average elevation in metabolism of 21 per cent. Corrected for changes in body temperature this figure becomes 16 per cent (see table 2).

4. This increased metabolism is brought about largely if not entirely by the thyroid gland (see table 2).

5. Nervous pathways through the superior cervical ganglia are not essential for bringing about stimulation of the thyroid gland by cold (see table 2).

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# THE BLOCKING AND DEBLOCKING EFFECTS OF ALTERNATING CURRENTS ON NERVE

A. ROSENBLUETH AND J. REBOUL

*From the Department of Physiology in the Harvard Medical School*

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The stimulating action of alternating currents (a.c.) on nerve has been extensively studied. Other effects of such currents, such as block of nerve impulses, changes of electrical excitability, of spike-potential magnitude and of conduction velocity, are only little known. Indeed, we are not aware of any systematic study of these problems since Wedensky's (1903) classical paper.

The present report deals with the block of nerve impulses produced by localized applications of a.c. and with the deblocking action which relatively weak a.c. is capable of exerting on fibers previously blocked by strong currents. In other publications to follow the other effects of a.c. mentioned in the preceding paragraph will be discussed.

METHODS. The observations were made on cats, anesthetized with dial (Ciba, 0.7 cc. per kgm. intraperitoneally). Two series of experiments were performed. In one the contractions of the gastrocnemius-soleus muscle were used as indicators of the passage of motor nerve impulses in the popliteal nerve. In the other the spike potentials of the A fibers in the excised peroneal nerve served as the indicators.

The mechanograms of the gastrocnemius were recorded semi-isometrically on a kymograph. Four or more shielded silver electrodes were placed on the popliteal nerve after section of the sciatic. Care was taken to maintain the blood supply of the nerve as intact as possible. Drills inserted into the tibia were used for fixation of the leg. The Achilles tendon was attached by a heavy thread to the short arm of the writing lever between the fulcrum and the point of application of one or more heavy rubber bands against which the muscle pulled.

The peroneal nerves used in the second series of observations were dissected in the thigh down to the point where the nerve begins to branch at about the head of the fibula. The excised nerves were placed in a moist chamber on 6 to 10 chlorided silver wires used as electrodes. The temperature of the chamber varied between 25° and 35° in different experiments, but was fairly constant in any given experiment.

The spike-potentials of the excised nerves were led off to an amplifier

monophasically, after crushing one of the extremities of the nerve. The amplifiers were either d.c. or capacity-coupled. The amplified signals were either photographed from a cathode-ray tube or recorded on tape by an ink-writing moving-coil oscillograph.

In the experiments in which the contractions of the gastrocnemius were used as indicators of conducted nerve impulses, the stimuli were supramaximal condenser discharges (1 to 4 v., 0.01 to 0.1$\mu$ farad). The excised nerves were stimulated by amplified pulses from a photoelectric cell, illuminated briefly 30 times a second; the shocks were rendered diphasic by a transformer.

The sources of a.c. used were two beat-frequency oscillators. In the early experiments the O.BR model of the Clough Brengle Company was employed. The range of this oscillator is from 30 to 16,000 cycles per second. The signal was amplified by a capacity-coupled amplifier. A voltmeter gave the output voltage for any frequency. In later experiments a General Radio Company type 713B oscillator with a range of 20 to 40,000 cycles per second was used. Both oscillators yielded fairly pure sine waves at all the voltages used, as verified with the cathode-ray oscillograph. The intensity of the a.c. was measured by means of a Weston thermocouple milliammeter, model 425—this meter is independent of frequency.

Results. I. *General considerations.* The responses of a muscle to maximal single shocks delivered to a pair of electrodes on the motor nerve may be reversibly modified in several ways after the application of a.c. to the nerve through another pair of electrodes placed nearer to the muscle. Figure 1 illustrates the different modifications of the muscular responses which can take place: immediate increase (A); immediate decrease (B); delayed decrease (C); immediate decrease followed by a delayed increase (D).

Some of the changes may be of muscular origin. For instance, the increase shown in figure 1A could be attributed to a neuromuscular posttetanic enhancement (Schiff, 1858; cf. Rosenblueth and Morison, 1937). A similar increase occurs when the a.c. is applied to electrodes placed farther, instead of nearer to the muscle—a situation in which the motor nerve impulses do not traverse the region of the nerve directly acted upon by the a.c.

In order to separate the muscular phenomena from those occurring in the nerve as a consequence of the application of a.c., in many experiments three pairs of electrodes were used on the motor nerve. Maximal single test shocks were delivered to the far and near pairs (with respect to the muscle) and a.c. was sent through the middle pair. In such conditions, the differences between the responses to the test shocks at the distal and proximal electrodes were due to the fact that only one of the

test nerve volleys had to go through the region treated by a.c. Such differences permitted, therefore, the evaluation of the action of the a.c. upon the nerve impulses. In the experiments in which the nerve action potentials were recorded, instead of the muscular responses, there was, of course, no question about any non-nervous factors.

After elimination of the muscular phenomena by the methods indicated the effects of a.c. upon the nerve were found varied and complex. In a given animal several or all the results illustrated in figure 1 could be obtained, depending upon the intensity and frequency of the a.c., upon the interelectrodal distance, and upon how long the current was applied.

Because of the number of factors involved, uniformity of results was seldom obtained. For instance, the law correlating the duration of the

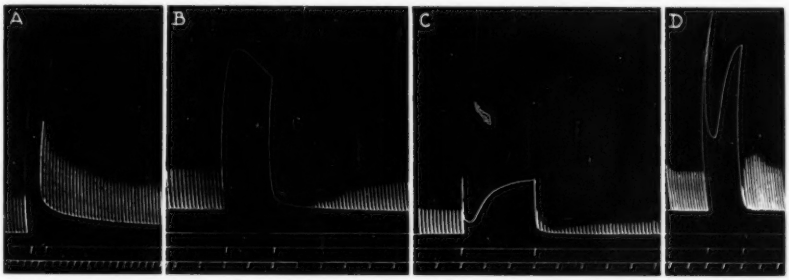


Fig. 1. Effects of a.c. on the responses of the gastrocnemius. Two pairs of electrodes on the popliteal nerve cut centrally. Single maximal condenser discharges applied continuously at the rate indicated by the twitches to the electrodes distal to the muscle. Between signals a.c. applied to the electrodes nearer to the muscle. In this and the succeeding kymograph records the time signal indicates 5-sec. intervals.

A, frequency: 100. Voltage: 10. B, frequency: 100. Voltage: 20. C, frequency: 2,000. Voltage: 10. D, frequency: 500. Voltage: 10.

block of nerve impulses with the intensity of a.c. was qualitatively different for different frequencies or for the same frequency in different animals. Moreover, the same blocking current applied several times in succession in the same animals, even after full recovery of the muscular responses, had, as a rule, increasing effects (see fig. 7).

For these reasons the results reported below have led only to qualitative, not quantitative, conclusions.

II. *The blocking action of currents of various intensities.* The depression of the muscular responses after a.c. in figure 1B is due to a reversible block of the nerve impulses. The progressive recovery indicates that the block subsides at different rates in the several motor fibers of the nerve. When a more intense current was applied the block was more prolonged and the

gradual recovery slower. Although block as such denotes a critical step in the nerve—i.e., either a nerve fiber conducts or it does not—it is legitimate to speak of several degrees of block if the time for recovery is taken as a criterion of the magnitude of the blocking process. This criterion was adopted and the degree of block was measured by the time for recovery of a given fraction of the total number of motor fibers in the nerve—i.e., the time for recovery to an arbitrary constant magnitude of the response.

The impedance of nerve is not constant for a given frequency of a.c., but varies with the voltage applied. Cole (1936) has reported such variations for relatively low voltages. In the present experiments the total impedance of the nerves was calculated from the intensities of the current at the voltages employed. Figure 2 is typical of the results obtained.

Usually both voltage and intensity of current were measured. On the other hand, increasing the voltage led always to increased intensity. For qualitative purposes, therefore, either of the two measurements may be used.

Sufficiently low intensities of a.c. do not produce any block. As the intensity is increased a block appears, which usually increases in magnitude —i.e., number of fibers blocked—and duration until the intensity is such that irreversible changes occur in the nerve.

Before deciding that such irreversible changes have taken place it is necessary to ascertain that a sufficiently long wait will not result in recovery. Thus, in one experiment, after several applications of a.c., including currents as strong as 10 ma., not more than 5 per cent of the nerve fibers had recovered after a wait of over 30 minutes. Twelve hours later, however, the muscular response was tested and indicated practically 100 per cent recovery. This observation was made on an irrigated nerve; similar tests were not made upon excised nerves.

Figure 3 illustrates the relation between degree of block and intensity of current in a typical experiment. With two exceptions, out of 12 animals, all the cases studied showed a similar direct relationship.

In one of the exceptional animals a frequency of 500 per sec. was applied for 15 sec. As the intensity was increased up to 1.4 ma. the blocking effect increased. A further increase up to 4.6 ma. caused less block. Greater intensities (e.g., 9.5 ma.) produced a marked block, less, however, than that produced by 1.4 ma. These results were carefully controlled several times by increasing or decreasing progressively the intensity. The other exceptional animal showed similarly an increase, then a decrease and finally again an increase of the blocking effects of progressively increasing voltages at frequencies of 100, 1,000 and 16,000 cycles per sec.

III. *The blocking action of currents of various frequencies.* The impedance of nerve changes with the frequency of a.c. applied (Cole, 1936).

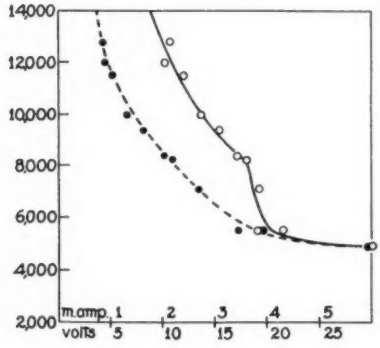


Fig. 2

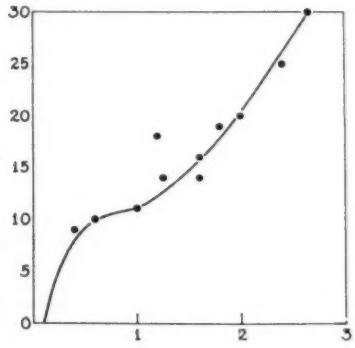


Fig. 3

Fig. 2. Changes of impedance of nerve with voltage or intensity. Frequency: 30 per sec. Ordinates: impedance in ohms. Abscissae: upper scale, ma.; lower scale, volts. Circles, full line: volts. Dots, broken line: ma.

Fig. 3. Block as a function of intensity. A.c. of frequency 1,000 was applied for 5 sec. at various intensities. Abscissae: intensity in ma. Ordinates: time in seconds after the end of the application of a.c. for recovery to ½ of the basal magnitude of the A spike potentials of an excised peroneal nerve stimulated continuously by maximal shocks at the rate of 30 per sec.

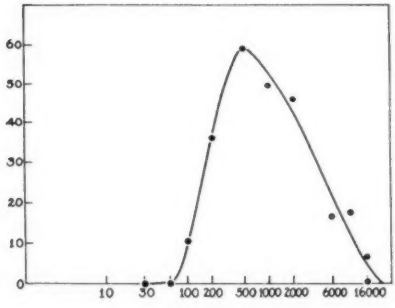


Fig. 4

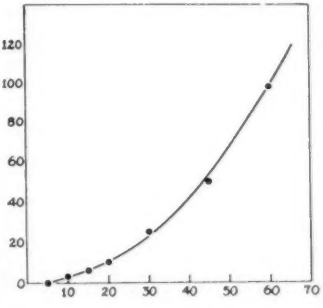


Fig. 5

Fig. 4. Unusual type of relation of block to frequency. A.c. with a constant intensity of 3.25 ma. was applied for 10 sec. at various frequencies. Abscissae: cycles per sec.; the scale is logarithmic. Ordinates: time in sec. after the end of the application of a.c. for recovery of the twitches of gastrocnemius to ⅔ of the basal magnitude.

Fig. 5. Block as a function of duration of a.c. Currents with a frequency of 1,000 and voltage 22 were applied for various durations. Abscissae: time of application in seconds. Ordinates: time in seconds after the end of a.c. for recovery of the twitches of gastrocnemius to ½ the basal magnitude.

In the present experiments the intensity of the current flowing through the nerve was kept constant by adjusting the voltage when the frequency was varied. Usually the blocking action of a.c. was practically independent of the frequency up to about 100 cycles per sec. With higher frequencies the blocking effect decreased rapidly. In a few instances, however, a maximal block was found at frequencies of 500 to 2000 c.p.s. Lower or higher frequencies of the same intensity produced briefer and less marked block (see fig. 4). Although in the majority of the animals frequencies higher than about 5000 per sec. were less efficient as blocking agents than frequencies lower than 1,000 per sec., it was always possible, by sufficiently increasing the intensity, to obtain as deep and prolonged blocks as desired with any frequency up to 40,000 per sec.

IV. *The blocking action of currents applied for various durations.* At any of the intensities (up to 10 ma.) or any of the frequencies used, a sufficiently brief application of a.c. did not result in any appreciable block.

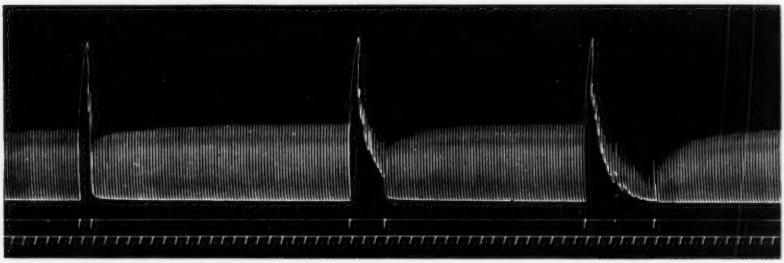

Fig. 6. Preparation and record as in figure 1. Between signals applications of a.c., frequency 16,000, intensity 5 ma.

For any given frequency of sufficient intensity, as the periods of application were increased, the degree of block, judged by the number of fibers affected and by the duration of the effects, increased first more slowly, then more rapidly. Figure 5 shows a typical curve correlating duration of a.c. applied with duration of block. In figure 6 are illustrated increased depths of block with more prolonged durations of a.c.

V. *The influence of interelectrodal distance upon block.* This factor was tested by selecting a constant frequency of a.c., applying it for constant periods of time and adjusting the voltage so that the intensity remained constant at different interelectrodal distances. Beginning with an interelectrodal distance of about 35 mm. and then progressively decreasing this distance, the block first increased in depth and duration to reach a maximum at distances of about 5 to 15 mm. for the peroneal nerve of the cat. Further decrease of the distance led to smaller blocking effects which reached a minimum when the current was applied transversely to the nerve.

VI. *Cumulative effects.* Under this heading is described the following phenomenon, which invariably occurred in the experiments. If a certain blocking current was applied and sufficient time was allowed for an apparently complete recovery of the responses, then a subsequent application of the same current resulted in a greater block than the first. Figure 7 illustrates a typical instance. Early in any experiment these cumulative effects were more prominent than after several reversible blocks had been obtained. This phenomenon was the most disturbing factor encountered for a quantitative analysis of the influence of the different variables upon the degree of block obtained.

VII. *Block during the passage of a.c. and delayed block.* Thus far the effects reported have referred to blocking of the nerve impulses immediately after the cessation of a.c. The behavior of the nerve during the passage of the current is usually masked by the responses set up by the

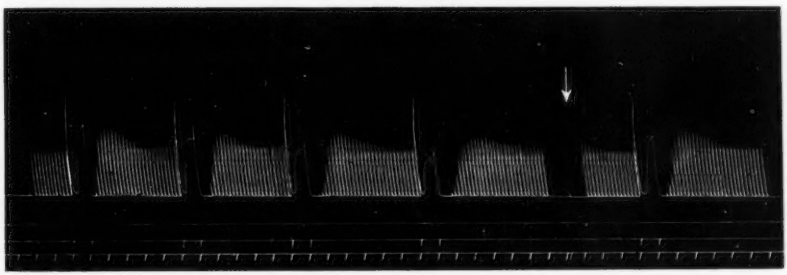

Fig. 7. Preparation and record as in figure 1. Between signals applications of a.c., frequency 1,000, voltage 14. The arrow denotes a pause of 1 minute.

blocking current. A block during the passage of a.c. may be readily seen, however, with high frequencies of stimulation (5,000 or more). The muscular contraction is a better indicator than the nerve action potentials of the responses of the nerve to a.c. of high frequency. With sufficiently high voltages the muscle contracted at the beginning of the application of a.c. but then relaxed promptly. The more intense the current the quicker this relaxation. Test maximal shocks applied above the a.c. did not lead to muscular twitches. Similar test shocks applied nearer to the muscle, on the other hand, yielded maximal muscular twitches, thus proving that the a.c. had ceased to stimulate the nerve and that it was blocking the nerve impulses coming from the more distant regions. Figure 8 illustrates a typical case.

Although usually the maximal blocking action of a.c. was seen at the end of its application, in certain observations the block was delayed i.e., the maximum took place some time after the current was stopped. A

typical case is that illustrated in figure 1C. Such delayed blocks were also observed in the experiments made with excised nerves, thus eliminating the intervention of neuromuscular phenomena.

VIII. *Deblocking action.* As Cattell and Gerard (1935; see also Cattell and Grundfest, 1935) have shown, when a high frequency a.c. is applied to a region of a nerve, if the intensity is adequate, the nerve may give a few responses and then cease to discharge. In these conditions it will usually be found that nerve impulses may pass through that region of nerve. This phenomenon was frequently encountered in the present

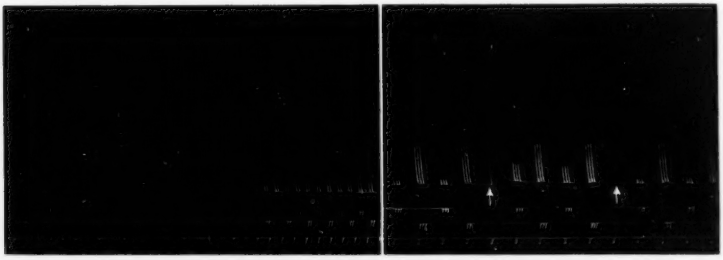

Fig. 8 Fig. 9

Fig. 8. Block during the passage of a.c. which does not stimulate the nerve. Three pairs of electrodes on the popliteal nerve. Upper signal: single maximal condenser discharges to the electrodes distal with respect to the muscle. Middle signal: similar maximal shocks to the nearer electrodes. Between the times indicated by the simultaneous upper and middle signals a.c., frequency 30,000, voltage 15, applied to the middle pair of electrodes.

Fig. 9. Deblocking effect during the passage of a.c. Three pairs of electrodes on popliteal nerve, far, middle and near to the muscle. Upper signal: single supramaximal condenser discharges applied to the far pair of electrodes. Middle signal: single supramaximal condenser discharges applied to the near pair of electrodes. The nerve had been blocked several times previously by a.c. to the middle pair of electrodes; most of the fibers were still blocked when this record was taken; this accounts for the difference in the test responses. Between arrows a.c. again delivered to the middle electrodes; frequency: 40,000; voltage: 9.

observations. The experimental condition may be described as an increased threshold to the a.c.—hence, no further responses of the nerve to this current; also the intensity is not sufficient to produce a block—hence, a passage of nerve impulses set up in other regions of the nerve. The intensity of the a.c. may be increased slightly so that some nerve fibers respond throughout the period of application, and yet nerve impulses may traverse the inactive fibers (see fig. 10B).

These observations were made on fresh nerves—i.e., nerves in which no fibers were blocked. It can be shown that weak a.c. not only permits the passage of nerve impulses but also exerts a deblocking action. This

deblocking action can only be detected, however, when nerves are tested in which some of the fibers are reversibly blocked by previous applications of relatively strong a.c. through the same pair of electrodes. The deblocking effects may occur only during the passage of weak a.c. or they may outlast the application.

To detect a deblocking action during the passage of a.c. it is necessary to use frequencies and intensities such that their excitatory effect is or becomes subliminal for all or the majority of the nerve fibers (fig. 9); otherwise the responses to the a.c. will interfere with those to the test shocks. The deblocking action which outlasts the application of a.c. (fig. 11) may readily be investigated without this precaution.

The magnitude of the deblocking action—i.e., the fraction of fibers deblocked and the duration of the reversibly renewed conduction—varied

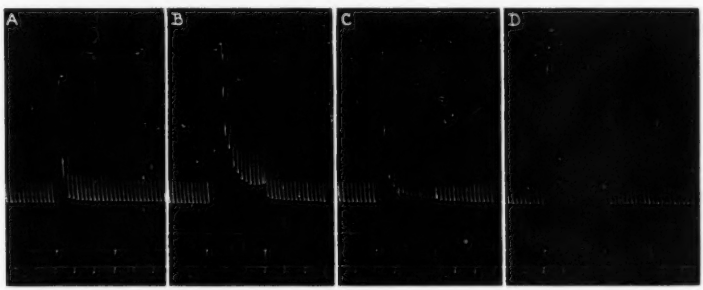


Fig. 10. Transition from deblock to block with increasing intensity of a.c. Two pairs of electrodes on popliteal nerve, far and near with respect to the muscle. Supramaximal condenser discharges delivered continuously through the far electrodes, as indicated by the twitches of the muscle. Several blocks had been applied to the near electrodes, so that about 50 per cent of the motor nerve fibers were blocked. Between signals a.c., 40,000 cycles, sent to the near electrodes with the following voltages: A, 14; B, 35; C, 80; D, 100.

with the intensity and frequency and with the time during which a.c. was delivered. In a series of observations, as the intensity was progressively increased the deblocking action augmented at first. Further intensification then resulted in less deblocking and the appearance of a delayed block. With stronger currents, finally, only block was apparent. Figure 10 illustrates a typical instance. With a frequency of 40,000, 5 volts or less showed no detectable action. With 10 volts clear deblocking appeared. In A and B are seen the effects of 14 and 35 volts. When 50 volts were applied an early deblocking was followed by a slight block. As shown by records C and D, with higher voltages the block became more and more prominent. At the end of this series 60 volts had only slight deblocking and a marked blocking action (see section VI).

The influence of frequency of the a.c. upon the deblocking action was qualitatively similar to that described in section III for the blocking effects. A given degree of deblocking occurred at lower voltages for low than for high frequencies.

The results of changing the length of the periods of treatment with a.c. were tested with weak currents—i.e., with intensities resulting only in restored conduction and no block. It was found that the fraction of fibers deblocked was usually the same with all durations used (2 to 30 sec.), but the deblocking after-effects of the current were much longer with the greater than with the briefer periods.

With moderate intensities of a.c. a deblocking action was frequently followed by block. Such blocks were sometimes delayed (fig. 1C), but

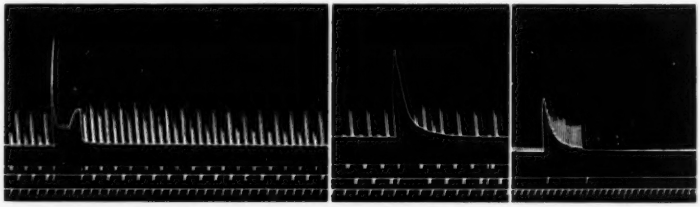

Fig. 11 Fig. 12 Fig. 13

Fig. 11. Deblocking effects outlasting the application of a.c. Three pairs of electrodes on popliteal nerve. Upper signal, maximal condenser discharges to the distant electrodes; and lower signal, similar shocks to the electrodes nearer the muscle. A large fraction of the motor nerve fibers had not recovered from block due to several previous applications of strong a.c. to the middle electrodes; this accounts for the difference in magnitude of the test twitches. The tetanic contraction of the muscle shows delivery of a.c., frequency 200, voltage 5, to the middle electrodes.

Fig. 12. Deblock followed by block during the application of a.c. Preparation and record as in figure 11. Between the points marked by simultaneous signals a.c., frequency 20,000, voltage 10, delivered to middle electrodes.

Fig. 13. Deblock during a.c. followed by block at the end of the current. Preparation and record as in figure 10. Many nerve fibers blocked by previous strong a.c. Between signals a.c., frequency 16,000, intensity 7 ma., applied to near electrodes.

they could also take place during the passage of the current (fig. 12) or suddenly at the end of this passage (fig. 13).

The deblocking effects were complicated in some instances by the phenomenon illustrated in figure 14. A.c. with a frequency of 30,000 and voltage 20 elicited a muscular response which rapidly subsided. The delivery of single maximal shocks to a distal pair of electrodes then led to the appearance of responses which were obviously tetanic in nature. Such tetani may be explained by assuming that the single nerve-volleys set up by the single shocks momentarily lower the threshold of the nerve to the a.c. continuously applied (Wedensky, 1903). This change in

threshold was found to depend on the intervals at which the single shocks were applied. The tetani were higher and more prolonged when the test shocks succeeded each other at relatively long intervals. Test shocks in rapid succession resulted in smaller and briefer tetani. Figure 14 was selected as the most outstanding example of this phenomenon. Similar tetanic responses were, however, clearly recognizable in other cases. They occurred not only when the test shocks were applied beyond the a.c. electrodes but also when single shocks were delivered nearer to the muscle.

The deblocking effect of a.c. was more readily demonstrable during the passage of the current than after cessation of a.c. When a large proportion of the fibers in the nerve had been deeply blocked by several applications of intense a.c. within a short period of time, no outlasting deblocking action could be detected. The best results were obtained when nerves were tested in which some fibers had been blocked by many moderate applications over a period of several hours—i.e., presumably when the

Fig. 14. Repetitive responses to single shocks during the passage of a.c. Preparation and record as in figure 10. Upper signal: single maximal condenser discharges to the far electrodes. Between the two middle signals a.c., frequency 30,000, 20 volts, applied to the near electrodes.

fibers to be deblocked were not too deeply blocked. In all instances the deblocking effects were reversible. They did not outlast the block used as a background for their demonstration.

Discussion. The data presented are not sufficient to justify any hypothesis as to how a.c. blocks nerve impulses. Certain negative statements which exclude some of the possible factors involved can, however, be made. Thus, it might be thought that block by a.c. could be due to the heating effects of the current. Indeed, Einthoven (1900) is often quoted as having stated that with a frequency of $10^6$ and an intensity of 10 ma., a.c. would raise the temperature of a nerve by 65° in 1 sec., and therefore kill it. It should be pointed out that Einthoven emphasized the fact that in his experimental conditions the intensity of the current would rapidly decrease to a small fraction of the initial value. Furthermore, Einthoven used very high voltages, over 2,000, which imply a much higher impedance than those found by Cole (1936). In the present obser-

vations, up to a frequency of 40,000, intensities of 10 ma. were obtained with less than 100 volts. Hence the resistance was much smaller than that quoted by Einthoven in his calculations, and consequently the heating effect proportionately smaller. The pertinence of these arguments is shown by the complete reversibility of all the results recorded.

Several considerations indicate that the thermal effects of the currents play probably only a negligible rôle in the production of block. *1.* Although the resistance of nerve decreases when the frequency increases, the intensity of current necessary to produce a given block had usually to be made about 6 times greater when the frequency was changed from about 100 to 40,000. Since the amount of heat produced by the current is proportional to the resistance and the square of the intensity, if equal block meant equal rise in temperature the resistance of the nerve would have to be about 36 times greater at a frequency of 100 than at a frequency of 40,000 per second. Such a change in resistance is not likely (see Cole, 1936). *2.* If a.c. should block by heating, then further applications should invariably lead to increased block. The deblocking effects described in section VIII show that such is not the case. *3.* In excised nerves, at temperatures lower than normal, heating the nerve should improve conduction. Yet it was found that a.c. of a given frequency and intensity yielded approximately equal degrees of block whether the excised nerves were initially at 20 or at 35°.

The influence of frequency upon the magnitude of the blocks leads us to infer that these blocks do not depend exclusively on the voltage, the intensity or the quantity of electric current applied. The frequency effect would suggest that changes in polarization should be the most important factor determining the block. The polarizing action of a.c. will be discussed in a later report. It may be stated, however, that the magnitude of the block does not depend upon the gross changes in the polarization of the nerve (cf. Bishop, 1932).

The influence of the interelectrodal distance upon the degree of block obtained for a given frequency, intensity and duration of a.c. (section V) suggests the following considerations. The facts known about the actions of d.c. upon nerve point to maximal effects at the regions in contact with the electrodes. Similar maximal influence at the electrodes may be assumed for a.c. Indeed, the changes in electrical excitability to be reported later support this assumption. If the further assumption is made that the blocking process is additive, then a relatively short interelectrodal distance would permit the summation at the interpolar region of the effects at the two poles. Some experiments were attempted to test these hypotheses. Nerves were placed on 8 electrodes (1 to 8). A.c. was applied from 3 to 8. The nerve impulses were recorded electrically from 6 to 7. It was found that a.c., which blocked for a certain time the

responses to stimuli delivered from 1 to 2, yielded similar records when the stimuli were applied from 4 to 5. Such experiments are, however, difficult to evaluate. As will be shown later, a.c. modifies not only conduction but also the electrical excitability and the magnitude of the spike potentials of nerve. A large decrease of the spikes might erroneously be taken for block. No experimental conditions were found which could circumvent these difficulties. With the present data it is probable that the blocking process is maximal at the a.c. poles.

The data suggest that the action of a.c. upon the conductivity of nerve is probably complex. At least two opposite effects have to be postulated: a depressing process responsible for block and an enhancing process responsible for the deblocking action. In order to account for the facts it is further necessary to assume that these two processes are independent—i.e., that they do not interact directly but that they may coexist and subside with different time courses.

On the basis of these assumptions some of the apparently contradictory effects encountered may be explained. Thus the delayed blocks (fig. 1C) would indicate an initial balance of the two influences and a more rapid subsidence of the deblocking than of the blocking process. A similar explanation, with an initial preponderance of the deblocking factor, applies to the records of figures 12 and 13, in which block succeeds deblock.

As stated in section II, although block of a nerve fiber can only appear as a critical step—either the fiber conducts or it does not—, the data indicate that the process responsible for interrupted conduction is a continuous function of the diverse characteristics of the a.c. employed. This view implies that both subthreshold and suprathreshold blocks may occur. The depth of the block above threshold is indicated by the duration of the block. The concept of a subthreshold blocking process furnishes an immediate explanation of the cumulative effects of a.c. (fig. 7). Successive applications of current would find conducting fibers in which the blocking process from the previous applications had not entirely subsided.

A similar interpretation is adopted for the deblocking process. Any application of a.c. develops this process to a variable extent. If fresh nerves—i.e., with no blocked fibers—are tested, the deblocking action would be unnoticed, although present.

In conclusion, it may be emphasized that the terms "blocking process" and "deblocking process," as used in this discussion, are meant to have exclusively a physiological connotation. The two processes may be complex; indeed, some experimental data to be reported later suggest such a complexity. As regards the physico-chemical events underlying these processes, the present lack of knowledge of the properties of nerves would render any speculation fruitless.

SUMMARY

The effects on nerve conduction of applications of alternating current (a.c.) to motor nerves were studied in cats. The indicators of nreve conduction used were either the mechanical responses of the gastrocnemius-soleus muscle or the spike-potentials of the A fibers of the peroneal nerve.

Nerve impulses may be blocked after the application of a.c. The block is a function of intensity (section II), frequency (III), duration (IV) and interelectrodal distance (V). Repeated applications of a.c. lead to increasing blocks (VI). Block may be demonstrated during the passage of a.c. (VII); it may be delayed (VII).

A.c. has a deblocking action which can be demonstrated after some fibers have been blocked. This action is an independent process, not a subsidence of the previous block (VIII).

In the discussion it is shown that applications of a.c. lead to the appearance of both the blocking and deblocking processes. The two are independent and therefore coexist. Their time course of subsidence is different. The presence or absence of conduction in the fibers of a nerve treated by a.c. depends on the resultant of the two opposite effects.

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# CONDITIONED REACTIONS TO POSITION AND ANGULAR ACCELERATION

E. A. SPIEGEL AND M. J. OPPENHEIMER[1]

*From the Department of Physiology and the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa.*

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The question of connection of the labyrinth with the cerebral hemispheres was studied in previous papers by various methods (stimulation of the labyrinth after localized strychninization of cortical areas, recording of the cortical potentials before and after the rotation of animals, Spiegel (1932–34), and Spiegel and Price (1937)).

Since these experiments indicated that labyrinthine impulses may reach the cerebral hemispheres and even the cerebral cortex, the question arose as to which functions may be performed by these vestibulo-cerebral mechanisms. The labyrinthine placing reaction of the forelegs, occurring when cats are lowered, head down, with the eyeballs enucleated, depends, according to Bard and Orias (1933), on the frontal and sigmoid gyri being intact. Thus, the cerebral cortex may participate in some primitive reflex functions of the labyrinth. One has, however, to bear in mind that the labyrinth not only influences, by simple primitive reflex mechanisms, posture and equilibrium, but is one of the most important receptors for conscious phenomena, i.e., spatial orientation, perception of position, and movements. In order to approach the study of the part played by vestibulo-cerebral mechanisms in these functions, the study of conditioned reflexes seemed a promising method.[2]

Therefore in the present series of experiments it was studied whether conditioned reflexes may be developed, in which the conditioned stimulus is represented by certain positions or by angular acceleration, and an effort was made to ascertain whether the labyrinth plays a part in these reactions.

[1] Aided by a grant from the National Research Council to E. S.

[2] Although a crude analysis, for instance of auditory and visual stimuli still may be found in decorticated animals (Pavlov (1927) and his school, Ten Cate (1934) Poltyreff (1936)), the finer analysis of such stimuli depends on the integrity of the cerebral cortex. The conception of Pavlov therefore still seems to be justified that the cerebral cortex should be regarded as the essential organ for the maintenance and establishment of conditioned reflexes.

I. CONDITIONED REACTIONS TO POSITION. *Method.* Dogs were slowly rotated on a position table similar to that used on patients by Grahe (1932), from a sloping position (head 30° or 25° above or below the horizontal plane, into another oblique position 5°–25° below or above the horizontal plane. When the horizontal plane was passed the unconditioned stimulus, an electrical shock was applied but only during the motion in one direction, upwards or downwards, while this stimulus was omitted during the return movement to the original position. Head, body, and extremities[3] were fastened by appropriate holders with as little discomfort to the animal as possible and in such a way that changes in position, except the desired one, and particularly sliding of the animal in an inclined position, were prevented. The head and face of the animal were covered by a cap preventing vision so that retinal impulses could not play a rôle in the development of the reactions studied. A vertical hard rubber disc, the center of which coincides with the transverse axis, is fixed on the side of the rotating table so that it participates in the rotation. It passes by a vertical hard rubber bar fixed on the frame that holds the axis of rotation. Three rows of contacts on the rotating disc and on the vertical bar respectively close three independent circuits: One records the position of the rotating table in relation to the horizontal plane in 5° intervals, the second records the moment when the board passes through the horizontal plane, the third applies an electrical shock to the fore or hind leg and records this stimulation. Besides, respiration and the defense reactions of the stimulated leg were recorded. In order to avoid conditioning based on time intervals between the beginning of rotation and the application of the shock, the speed of rotation was frequently varied.

*Results and comments.* The experiments were purposely arranged in such a way that the unconditioned stimulus was applied in the most usual position, i.e., with the animal in the horizontal plane. Thus, the animals showing in the beginning of the experiments defence reactions (struggling, increased rate and amplitude of the respiration) in the extreme oblique position had to become accustomed to these abnormal positions. This was possible without too many difficulties so that the defence reactions in these positions became diminished and eventually subsided. Conditioned reactions could be developed in some animals even in the first of a series of experiments, the shortest number of reinforcements necessary to develop a conditioned reaction in or close to the horizontal position being 13 repetitions of combined unconditioned and conditioned stimuli; in some animals, however, it was several days until such conditioning took place. After they had been developed, the conditioned reactions were retained by the animals up to several weeks, so that they were still present at the beginning of a new experiment without reinforcement.

The reactions often did not appear in the horizontal position, but at 10° or 15° before this position was reached (fig. 1), so that the beginning of the

[3] It proved important, particularly in male animals, to fasten the hind legs, not in an extended but in a flexed and adducted position, since in extended position the animals became very restless and excited, so that the development of conditioned reflexes was rendered very difficult.

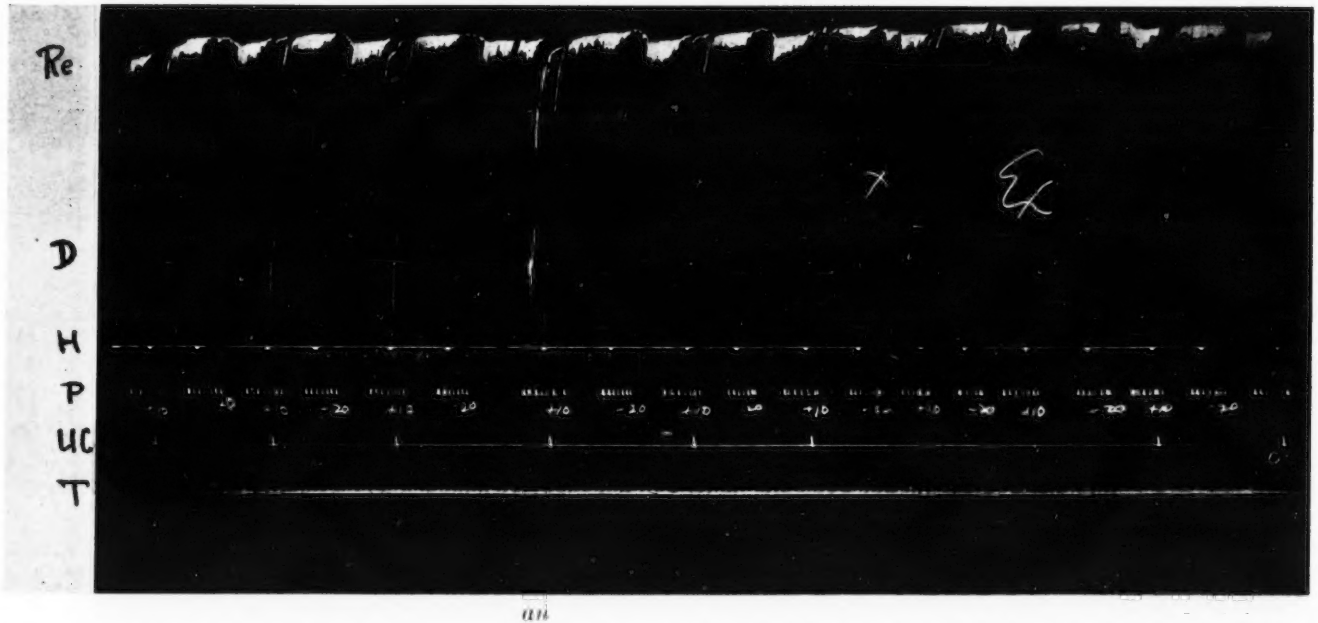


Fig. 1. Development of conditioned reactions to position. *Re*, respiration; *D*, defense reaction of the leg; *UC*, unconditioned stimulus (electric shock); *P*, position of animal in relation to horizontal plane; [between 10° above (+10°) and 20° below (−20°) the horizontal position]. *H*, horizontal position; *T*, time in seconds; an, anticipatory reaction; *X*, conditioned reaction; *Ex*, extinction.

conditioning could be recognized without interrupting the conditioning process. Also, the beginning of the motion from extreme oblique toward the horizontal position sometimes elicited defence reactions that appeared alone or combined with premature or exact conditioned reflexes. In interpreting these observations one has to consider several factors. The development of analysis of external agencies by means of conditioned reactions is always preceded by what Pavlov called a "period of generalization," and it seems possible that not only the horizontal position, but also other positions may, in the first stages of the experiments, become conditioned stimuli. Yet premature reactions appeared not only in the beginning of the conditioning process, but also after conditioned reactions were well developed. Furthermore, if generalization of stimuli were the only cause of incorrect reactions, such reactions should have appeared just as often before as after the horizontal position was reached. They appeared, however, much more frequently before the horizontal position than after it had been passed. This also refutes the assumption that these apparently incorrect reactions are due to low sensitivity of the receptor mechanism.

It is furthermore well known that the time relationship between the conditioned and unconditioned stimulus plays an important rôle in the development of conditioned reflexes, the circumstances for development of conditioned reflexes being optimal, if the conditioned stimulus precedes the unconditioned one. In applying this in our case, one has to consider that the animal was stimulated by an electric shock in the horizontal position; thus the optimal position for the establishment of a conditioned reflex was not the horizontal position itself, but a position reached shortly before the horizontal position. According to Wolfle's (1930) studies in auditory reflexes, the optimal time interval between conditioned and unconditioned stimuli is 0.5 second. Since we usually rotated the animal board by about 1° per second, a conditioned reflex should most easily be established not exactly for the horizontal position but for a nearly situated position of about $\frac{1}{2}$ to 1 degree before the horizontal position was reached. Thus this factor can only account for such premature reactions that appear quite closely to the horizontal position; it does not explain reactions that appear 10 to 15 degrees before the horizontal position or even in the beginning of the deplacement of the animal board from the oblique toward the horizontal position. Since all the factors hitherto considered do not explain the premature appearance of the conditioned reactions, and since it is known that conditioned reactions are quite frequently anticipatory in nature (Hull, 1937), it may be inferred that here we have to do with a type of conditioned reaction showing anticipatory characteristics. The biological importance of such a tendency to respond in

advance of injury was emphasized by Hull. Besides the premature reactions or, combined with them, reactions in exactly the horizontal position may also appear (fig. 1).

The purpose of these experiments was to condition not only certain positional stimuli but also stimuli depending on the motion in a certain direction. Thus, the unconditioned stimulus was applied in the horizontal position during the motion in one direction (up- or downward) only, but was omitted during motion in the opposite direction. In the first stages of the training, the conditioned reactions appeared in or close to the horizontal plane, not only during the motion that was previously accompanied by the unconditioned stimulus, but also during the motion in the opposite direction. In this stage of the conditioning process apparently only the position stimuli, but not the stimuli associated with the motion in a certain direction, were conditioned. Several times the seemingly paradoxical observation was made that after a series of paired conditioned plus unconditioned stimulations, e.g., during downward motion, the next upward motion was accompanied by a conditioned reaction in the horizontal position, while no conditioned reaction appeared at the subsequent downward motion. This may be interpreted by assuming that a weak conditioned reaction upon the horizontal position appeared during the upward motion, which disappeared due to extinction at the next downward motion.

While the conditioning of the horizontal position developed rather quickly, (on first day usually) most of the animals needed a longer time (about 3 days) to develop a differentiation of the two directions of motion. Eventually this discrimination was accomplished, and some dogs learned so well to distinguish the two directions that they still reacted in that direction only, that was in former trials associated with the unconditioned stimulus, even if the order was reversed and the unconditioned stimulus was applied only during motion in the opposite direction. Thus not only a certain position, but also the combination of this position with a uniform motion in a certain direction (up- or downwards) could be conditioned.

The inference that we are dealing with conditioned reactions is based upon the facts that these reactions appeared only after the previously neutral static and kinetic stimuli were repeatedly combined with unconditioned stimuli, and that anticipatory reactions as well as the typical phenomena of external and internal inhibition could be observed. Thus adventitious stimuli such as noise had a definite inhibitory effect, and the phenomenon of extinction could also easily be demonstrated, the conditioned reactions becoming diminished and disappearing if the conditioned stimuli were applied several times without reinforcement by the unconditioned one (fig. 1). As to the strength of these conditioned reactions,

one may observe in the anticipatory as well as in the correct reactions that they often do not reach that of the unconditioned reaction, while in some instances they may even exceed that of the conditioned ones.

The conditioned reaction to position may still be elicited after combined severance of the posterior columns and dorsal spinocerebellar tracts at the first or second cervical segments on the one hand, and after elimination of both labyrinths on the other hand. After the latter operation, however, it took a longer time than after the combined chordotomies until we obtained discrimination between the direction of the motion (up or downwards) associated with application of the shock in the horizontal position, and the opposite motion. Eventually, however, this discrimination was possible in the labyrinthectomized animals also. Also, after combining the chordotomies with the elimination of the labyrinthine impulses conditioned reactions to position as well as discrimination of up- and downward movement reappeared so that impulses carried by the ventral spinocerebellar tract and the spinothalamic system may eventually be sufficient for the development of these reactions.

II. Conditioned reactions to angular acceleration. *Method.* The dogs were rotated around a vertical axis in clockwise or counterclockwise direction first at a slow, constant speed (16.5° per second) that then passed smoothly, after a variable number of rotations (from 2–15) into rotation with acceleration. During this accelerated rotation the unconditioned stimulus, an electric shock, was applied to a leg. The cable connecting the motor and its system of gears with the turntable is conducted over 4 pulleys (two on the floor, two on the ceiling) so that it forms a loop like an inverted U. During the rotation at constant speed the experimenter holds a weight as close to the ceiling as possible by a cord that is conducted over a 5th pulley in the ceiling. In order to produce an accelerated rotation the experimenter releases the cord allowing the weight to fall, and then closes the circuit for electrical stimulation of the leg. At the moment the weight starts to fall a differential clamp with 2 excentric cams inserted above the weight grasps the cable at the highest part of the descending limb of the loop, so that the falling weight produces an accelerated rotation of the turning table; the action of the motor upon the turning table is eliminated at the moment when the part of the cable between the weight and motor is no longer taut. The acceleration may be increased by additional weights, or decreased by hanging counterweights on the ascending limb of the loop. A contact on the turning table connected with an electromagnetic signal marker is automatically closed with each turn, so that the time intervals between the rotations can be measured and the speed or acceleration of the rotation can be computed. The wires as well as the tubings connecting the rotating table with the signal magnets and recording tambours respectively are conducted below or above the rotating board in the axis of rotation; they are divided into a rotating and fixed part that remain in contact with each other during rotation. Thus the electric stimulation of the animal, recording of this stimulation and of the reactions of leg and respiration are not interfered with by the rotation. Before the conditioning was started and the accelerated rotation was accompanied by the electrical stimulation, the dogs were of course accustomed to the rotation, so that they reacted by leg movements neither to rotation at constant speed nor to the accelerated rotation.

*Results and comment.* Conditioned reactions to accelerated rotation (fig. 2) were developed with more ease than those to position in the preceding series of experiments. The minimal number of trials necessary for conditioning of accelerated rotation was 4, the maximal 25, the average 15. In all animals it was possible to establish these conditioned reactions in the first series of combined application of conditioned and unconditioned stimuli. After repeating the experiments for four days, the conditioned reaction to accelerated rotation was retained for up to $4\frac{1}{2}$ weeks. Similarly as in the conditioning of position the development of conditioned reactions could often be observed by the appearance of anticipatory reactions. Repetition of the conditioned stimulus for several times (2–4) was followed by the extinction of the conditioned reaction, which could be restored by 1 to 2 paired trials. In order to determine the threshold value of acceleration necessary to elicit these condi-

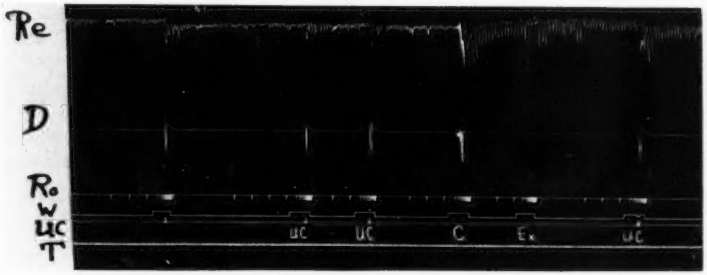


Fig. 2. Development of conditioned reactions to angular acceleration; *Ro*, rotation of turntable; *W*, fall of weight producing accelerated rotation; *C*, conditioned reaction. Other signs same as in figure 1.

tioned reactions, they were first developed at an acceleration of 8°/sec², then the acceleration was gradually reduced. In this way it was possible to obtain conditioned reactions at an acceleration of 2° 3°/sec². It is quite possible that the threshold of the reaction is still lower; unfortunately we could not further lower the acceleration without making the rotation irregular (apparently due to the unavoidable friction, particularly of the cable on the pulleys). We must, therefore, content ourselves with the statement that the threshold of the conditioned reactions to accelerated rotation is not above 2 3°/sec² and possibly lower.

It was rather easy to develop discrimination between rotation to left and to right. Counterclockwise accelerated rotation was, for instance, paired with an electric shock, while this stimulus was omitted on clockwise rotation (fig. 3). It was found that after 15 to 25 trials the counterclockwise rotation elicited the conditioned reaction, while the rotation

in opposite direction was not accompanied by the conditioned reaction. Discrimination of direction was usually preceded by a period during which CR occurred in both directions.

*Analysis of receptor mechanisms.* Optic reflexes were excluded by covering the eyes with a hood. Stimulation of the body surface by air currents arising during rotation around the body was avoided by covering the body. Yet it was found that the results were the same whether the body was covered or not. The external auditory meatus was plugged by cotton to reduce as much as possible the effect of slight acoustic stimuli due to the motion of the cable and the rotation of the turn table. Repeating the whole procedure (running the motor, releasing the weight) so that all parts were rotated except the animal board had no effect in animals with well developed conditioned reactions, indicating that slight

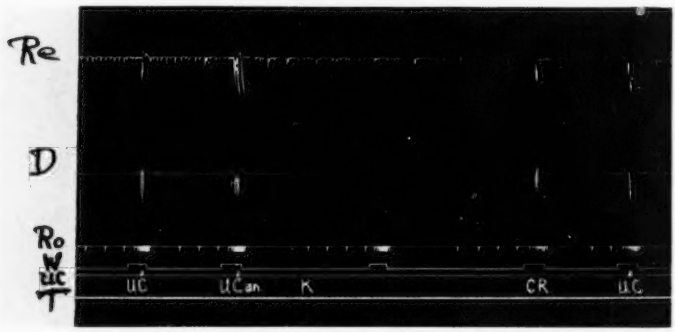


Fig. 3. Discrimination of direction. Animal is rotated in counterclockwise direction, only at *K* in clockwise direction; an, anticipatory reaction; *CR*, conditioned reaction. Other signs same as in figures 1 and 2.

acoustic stimuli evoked by these procedures were not the cause of these reactions. The inference that these conditioned reactions are not caused by acoustic stimuli is also proved by the experiments in which the animals were trained to react to rotation in one direction only, the rotation in the opposite direction, accompanied by the same acoustic stimuli, being without effect. These experiments also exclude the possibility that these conditioned reactions may be due to sensations from the viscera evoked by vestibulo-visceral reflexes. In order to avoid movements of the head or of parts of the body other than the pure rotation, head, body, and limbs were fixed as well as possible. Furthermore, dogs with the posterior and dorsal spinocerebellar tracts cut at the first or second cervical segment developed these reactions without difficulty.

Leiri (1927) has expressed the opinion that rotational sensations in man

do not originate directly in the labyrinth but are due to impulses from the skeletal muscles, the state of contraction of which is changed by the stimulation of the labyrinth. The fact that conditioned reactions to angular accelerations may be elicited after section of the posterior columns and of the dorsal spinocerebellar tracts indicates that one cannot accept such a view regarding the mechanism of these conditioned reactions, and makes it rather improbable that Leiri's theory holds true for rotational sensations in man.

In view of Bárány's (1911) assumption that rotational sensation depends on the nystagmus that is evoked by stimulation of the labyrinth, we studied in further experiments whether paralysis of the proprioceptive fibers originating in the external ocular muscles prevents the development of conditioned reactions to accelerated rotation. A 1 per cent procaine solution was injected into the orbit around the eyeball (1 cc. each medially, laterally, above and below the eyeball, 4 cc. total into each orbit). Such an injection did not interfere with the development of conditioned reactions to accelerated rotation, nor did it prevent the discrimination of direction. Conditioned reactions to accelerated rotation could also be developed when after section of the posterior columns and dorsal spinocerebellar tracts not only the extraocular muscles but also the facial muscles and muscles of mastication supplied by the trigeminal nerve were injected with 2 cc. of 1 per cent procaine on either side. One could eventually suspect that the dogs reacted to the time interval from the beginning of the rotation to the application of the unconditioned stimulus. This objection is invalidated by the fact that the number of rotations at constant speed preceding the accelerated rotations was widely varied during all experiments.

After bilateral labyrinthectomy[4] conditioned reflexes to accelerated rotation could not be obtained for a period from $\frac{1}{2}$ to 2 months. Then on regular training (every 2 to 3 days) a restitution of these functions may gradually be obtained in the following order. First conditioned reflexes appear at high accelerations only (above $10°/sec^2$) and are absent at low acceleration.[5] Discrimination of direction cannot be produced at this stage. Then CR appear on low acceleration also, but without discrimination of direction. Eventually discrimination of direction at high acceleration is obtained, while it remains absent at low acceleration. (Observed for 6 months after the 2nd labyrinthectomy.) Thus, discrimination of direction of rotation in the horizontal plane at low acceleration seems an important criterion of the analysing function of the labyrinth.

It may be of interest to compare with these experiments observations

[4] These animals had on repeated examination on a Bárány chair no nystagmus and a negative statie adaptation test (Rademaker) on a tilting table.

[5] Our preliminary note (This Journal **123**: 192, 1938) corresponds to this stage.

on patients with unexcitable labyrinths. M. H. Fischer (1925) describes a patient suffering from postneuritic degeneration of the eighth nerves. This patient recognized the usual rotation of the Bárány chair apparently due to the slight vibrations associated with the rotation and the effect of the centrifugal force upon parts of the body outside the labyrinths. He failed to notice the rotation when it was very gradually and carefully started. He recognized the direction of rotation on fast starting rotation, but made grave errors when the rotation started slowly. Thus, it seems that the results of our animal experiments are in good agreement with observation from the human pathology. It would, however, be desirable to have Fischer's single observation checked on larger material.

## SUMMARY

1. Conditioned reactions to position showing typical phenomena of other conditioned reactions (anticipatory reactions, external and internal inhibition) were developed in dogs. Not only a certain position (horizontal position) but also the combination of this position with a uniform motion in a certain direction (around a horizontal axis, upwards or downwards) could be conditioned. The receptor mechanism of these reactions is of complex nature. Not only the labyrinth, the posterior columns and the dorsal spinocerebellar systems play a part, but one may still obtain these reactions after elimination of these sensory mechanisms; they are then apparently elicited by way of remaining ascending tracts of the spinal cord.

2. Conditioned reactions to angular acceleration on rotation around a vertical axis are easily obtainable. Their threshold lies at or below 2-3°/sec$^2$. The dogs were also trained to discriminate the direction of the accelerated rotation. The receptor mechanism of these reactions was analyzed. While the loss of the labyrinths may be compensated as far as the development of conditioned reactions to angular acceleration is concerned, the discrimination of direction of rotation around a vertical axis remains absent in labyrinthectomized dogs on rotation at low acceleration. Discrimination of direction of rotation in the horizontal plane at low acceleration seems therefore an important criterion of the analyzing function of the labyrinth.

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# THE EFFECTS OF PREGANGLIONIC DENERVATION ON THE SUPERIOR CERVICAL GANGLION

A. ROSENBLUETH AND W. B. CANNON

*From the Department of Physiology in the Harvard Medical School*

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In a previous publication (Cannon and Rosenblueth, 1936) observations were reported which led us to conclude that degeneration of the cut preganglionic fibers of the cervical sympathetic increases the sensitivity of the superior cervical ganglion to acetylcholine. This conclusion has been questioned by Brücke (1938). From his observations he inferred that the only effect of preganglionic denervation on the ganglion is to lower the limit of concentration at which acetylcholine ceases to have an excitatory action on the ganglion cells, and becomes instead a depressant agent.

In the same publication Brücke reported observations bearing on the sensitization to adrenaline of the preganglionically denervated nictitating membrane. Although the responses of the chronically decentralized side were usually greater than those of the control acutely decentralized membrane of the other side, as long as the contents of the orbits were intact, removal of the eyeballs abolished this difference. He concluded, therefore, that the smooth muscle of the nictitating membrane is not significantly sensitized by preganglionic denervation, and that the greater responses of the operated side when the eyeballs are intact are to be attributed to a sensitization of the smooth muscle elements responsible for protrusion of the eye when adrenaline is injected.

The discrepancies between some of Brücke's observations and conclusions and our own, and the important bearing of these facts and inferences on the problem of sensitization of denervated structures, prompted the present study.

METHODS. Cats were used, anesthetized with dial (Ciba, 0.7 to 0.8 cc. per kgm., intraperitoneally). Under ether the cervical sympathetic was cut aseptically on one side 9 to 25 days before the observations. A tracheal cannula was inserted, the adrenal glands were ligated, and the intact cervical sympathetic was cut.

Isotonic contractions of both nictitating membranes were recorded with as similar conditions as possible—i.e., the two orbits were on a horizontal plane; the serresfines tied to the silk threads leading to the levers were clipped symmetrically on the membranes; the levers were adjusted to insure

the same load (about 5 grams) and magnification (about 15-fold). When only intravenous injections were given the head was in the prone position and the contractions were recorded directly, without pulleys. When acetylcholine was applied locally to the ganglia or when intracarotid injections were made, the animals lay supine. Pulleys placed symmetrically were then interposed between the serresfines and the levers.

The threads connecting the serresfines with the recording levers were interrupted at a convenient place by a fine wire hook-and-ring link, which permitted the membranes to be detached from and reattached to the recording system without modifying the insertion of the serresfines. It was thus possible to determine accurately changes in the responses to adrenaline or acetylcholine caused by diverse experimental procedures: excision of the superior cervical ganglia, emptying or removal of the eyeball, section of the conjunctiva and deep layer of smooth muscle, severance of the striated ocular muscles.

For exposure of the ganglia in the local application experiments the usual procedure was to dissect carefully the ganglia free of fascia through a midline incision and to expose them when necessary by moderate lateral pull of the sterno-mastoid muscles by weighted retractors. In a few instances Brücke's suggestion was followed of removing the trachea and esophagus.

The substances injected were the following: adrenaline (adrenalin chloride, Parke, Davis and Company); acetylcholine (chloride, Merck); curare (the crude Brazilian product); atropine (sulphate, Merck).

Results. I. *The sensitization of the nictitating membrane to adrenaline by preganglionic denervation.* The withdrawal of the nictitating membrane which occurs upon injection of adrenaline is mainly due to a contraction of the smooth muscle elements inserting in that structure, and probably also partly due to protrusion of the eyeball (Rosenblueth and Bard, 1932). Protrusion of the eyeball may be produced by contraction of the deep layer of smooth muscle in the orbit. When, on the other hand, the striated muscles of the eye contract, enophthalmos results, and consequently the membrane is moved outward, passively, over the conjunctiva.

Removal of the eyeballs not only eliminates the possibility that exophthalmos or enophthalmos may passively influence the membrane, but the operation may also sever or destroy some of the smooth muscle elements which directly and actively withdraw it. The distribution of this smooth muscle in the cat's orbit has only recently been studied (Acheson, 1938). The observations here reported were made in an attempt to determine the rôle which the several factors mentioned play in the responses of the nictitating membrane to adrenaline.

Emptying the eyeballs after partial excision of the cornea does not modify significantly the responses to adrenaline of the membrane, whether

acutely or chronically decentralized (fig. 1A and B). This procedure excludes the influence of exophthalmos or enophthalmos. Since the operation does not appreciably modify the responses it may be concluded that in the experimental conditions employed for recording the movements of the membrane passive effects may be discarded.

If after emptying the eyeballs they are later entirely removed, the responses to relatively small doses of adrenaline (1 to 10$\gamma$) are increased on the normal side and decreased on the denervated side. A decrease in the ratio of the responses from the two sides will thus occur, tending to cancel the discrepancy in magnitude interpreted as sensitization. It is noteworthy, however, that in our observations, unlike those reported by

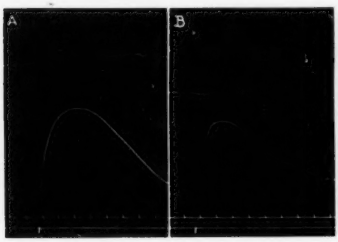


Fig. 1

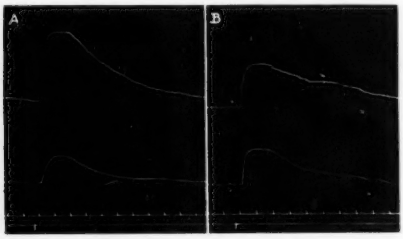


Fig. 2

Fig. 1. Negligible influence of emptying the eyeballs on the responses of the nictitating membrane to adrenaline. Upper record: left membrane (decentralized acutely). Lower record: right membrane (decentralized 16 days). Injections of adrenaline (2$\gamma$ intravenously) A, before, and B, after emptying the eyeballs.

In this and the succeeding figures the time signal denotes 30-sec. intervals.

Fig. 2. Influence of the dissection of the deep orbital fascia (surrounding the extrinsic eye muscles) on the responses of the membrane to adrenaline. Upper record: right membrane (decentralized 17 days). Lower record: left membrane (decentralized acutely). Injections of adrenaline (2$\gamma$ intravenously) A, before, and B, after separation of the deep fascia from the sclera without emptying or removal of the eyeball.

Brücke (*loc. cit*) only 3 out of 18 animals, without eyeballs, gave approximately equal responses from the two sides. In the other 15 instances the contractions of the preganglionically denervated membrane remained markedly greater than those of the normal control side (figs. 3, 4, 5 and 6).

The operative procedure followed in removing the eyeballs involves two steps which may affect the reactions of the membrane: the dissection of the deep fascia of the orbit, including the deep layers of smooth muscle, from the sclera, and the section of the eye muscles. The only step of importance in bringing about the changes in the membrane responses described in the preceding paragraph is the dissection of the deep smooth

muscle layer. This dissection, whether preceded by emptying of the eyeball or not, increases the contractions of the normal side and decreases those of the denervated side (fig. 2). If the extrinsic striated eye muscles are cut or the eyeballs are removed after dissection of the deep fascia, no further changes are noticeable in the responses of the membranes.

Severance of the striated eye muscles could only be important if adrenaline were capable of causing their contraction, as Duke-Elder and Duke-Elder (1930) have claimed. In four experiments, made for the purpose of testing this point, we obtained negative results. The Duke-Elders noted contraction only when these muscles were *in situ*, and, since they neglected to perform a complete denervation, central effects were not excluded.

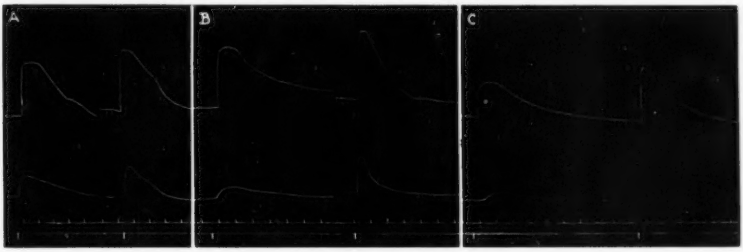


Fig. 3. Influence of atropine and curare on the responses of the membrane to adrenaline and acetylcholine. Adrenals ligated; superior cervical ganglia removed; eyeballs removed. Upper record: right membrane (decentralized 8 days). Lower record: left membrane (decentralized acutely).

A. Before atropine and curare. Intravenous injections of adrenaline (4$\gamma$) and acetylcholine (20$\gamma$).

B. In another similar preparation. After atropine (1 mgm. per kgm.). Adrenaline (2$\gamma$) and acetylcholine (2 mgm.).

C. Same animal as in A. After atropine and curare (a paralyzing dose). Adrenaline (2$\gamma$) and acetylcholine (2 mgm.).

They did not observe contractions when they tested the hormone on excised muscles, though these responded readily to acetylcholine. In the four cats which we studied, the muscles were denervated acutely; acetylcholine invariably caused responses, thus proving that the circulation was intact; adrenaline had positive effects only when the muscles had not been carefully freed from surrounding fascia and smooth muscle.

II. *The sensitization of the preganglionically denervated nictitating membrane to acetylcholine.* Rosenblueth (1932) showed that the nictitating membrane contracts in response to acetylcholine, and that this response is increased after degeneration of the postganglionic nerve supply. In the present observations it was found, similarly, that the responses of the preganglionically denervated membrane to acetylcholine are greater than

those of the normal control. The sensitization was still apparent after elimination of extraneous factors by removal of the eyeballs and the superior cervical ganglia.

The sensitization to acetylcholine is approximately equal to that to adrenaline when the tests are made without previous administration of any drug other than the anesthetic (fig. 3A). By equal sensitization is meant that doses of the two substances which evoke approximately equal submaximal contractions on the denervated side evoke also equal responses on the normal side.

Atropine, which antagonizes the action of acetylcholine on the membrane (Rosenblueth, 1932), has a greater effect on the denervated than on the control side. The relative sensitivity to acetylcholine after atropine is, therefore, as a rule, less than that to adrenaline (fig. 3B).

Curare, like atropine, antagonizes the action of acetylcholine on the membrane. The effect of curare appears, however, to be greater on the normal than on the denervated side. The relative sensitivity to acetylcholine after curare is, therefore, as a rule, greater than that to adrenaline (fig. 3C).

III. *Responses to intravenous injections of acetylcholine.* The direct action of acetylcholine on the smooth muscle of the nictitating membrane is muscarine-like. It occurs with much smaller doses than those necessary to elicit the stimulatory nicotine-like effect on the ganglion. It is not possible, therefore, to test the action of acetylcholine on the ganglion without the use of some drug or procedure which will diminish or eliminate the direct action on the membrane.

As is well known, atropine blocks greatly the muscarine-like actions of acetylcholine, while depressing only slightly the nicotine-like effects. For this reason both Cannon and Rosenblueth, and Brücke, used this drug in their observations. It should be emphasized, however, that a given dose of atropine does not abolish the muscarine effects entirely. They can still be evoked by sufficiently high doses of acetylcholine. The responses of the membrane to large doses of acetylcholine injected intravenously after atropine may, therefore, be composite, partly due to the ganglionic discharge, and partly to the direct action of the substance on the membrane. They might, indeed, be exclusively due to the latter component, without any ganglionic participation.

The only control which indicates the share of the ganglion in such responses is a comparison of the responses before and after removal of the ganglia. This control was used by Cannon and Rosenblueth, and their conclusions were thus justified. Brücke, on the other hand, does not report that he applied this control.

When they injected acetylcholine intravenously Cannon and Rosenblueth systematically used curare, in addition to atropine. In these

conditions they found that the responses of the chronically decentralized membrane to acetylcholine were greater than the responses to adrenaline when the contractions of the acutely decentralized control membrane to the two hormones were equal; or, conversely, that the responses of the control membrane to acetylcholine were smaller than to adrenaline when the responses of the operated side to the two agents were equal. These results were consistently confirmed in 6 animals in the present study.

In addition to these confirmatory observations a series of tests was made in which atropine, without curare, was employed. In these tests the eyeballs were always removed. Brücke reports equal sensitivity to adrenaline and acetylcholine in these conditions. We failed to confirm this report. In 3 out of 6 animals the responses of the denervated side to acetylcholine were smaller than those to adrenaline (equal responses of the normal controls). In another cat the action of acetylcholine was equal to that of adrenaline. In the last 2, with doses of acetylcholine smaller than 2 mgm., the denervated side was more sensitive to acetylcholine than to adrenaline, but with doses of about 4 mgm. precisely the reverse was found. In 3 of the animals curare was later injected, whereupon the results of Cannon and Rosenblueth, mentioned above, were confirmed. In the other 3 cats the ganglia were removed and acetylcholine given in the same doses as before. The responses were now only slightly smaller on both sides, the main difference being the disappearance of the initial sharp rise of tension present when the ganglia were intact. It may be concluded that the responses were largely due to the muscarine effect on the membrane. The results, therefore, are not sufficiently discriminative for an accurate analysis of the ganglionic events.

IV. *Responses to applications of acetylcholine locally to the ganglia.* Cannon and Rosenblueth reported that when in cats, which had received no other drug than the anesthetic, cotton pledgets soaked with Ringer's solution containing acetylcholine at various concentrations were applied locally, a marked difference of sensitivity was revealed between the chronically denervated and the control ganglion. Concentrations were found which, applied for a similar period of time (e.g., 30 sec.) to the two sides, elicited a marked response on the denervated side and had no effect on the control side.

These observations were consistently confirmed in the present study in 5 cats. Whether the eyeballs were intact, emptied or excised (fig. 4B), the phenomenon was present. The influence of the period of application on the responses was studied in two animals; the pledgets were left in contact with the ganglia some time (about 1 min.) after the start of the prompt response of the denervated side. In one cat the results were inconclusive. In the other, whereas a 1 per cent solution of acetylcholine applied for 30 sec. called forth a response on the denervated side and none

in the control membrane, the same solution applied for 90 sec. resulted in a delayed response on the control side also (fig. 4).

Applications of similar pledgets of cotton with the same solutions of acetylcholine, to the same region of the animal but after removal of the superior cervical ganglia, caused contractions of the membranes in the 4 animals which were tested. These contractions were, however, smaller and more delayed (i.e., longer periods of application were necessary) than those recorded when the ganglia were intact.

V. *Responses to intracarotid injections of acetylcholine.* Although the results of local applications of acetylcholine, reported in the previous section, seem to furnish clear evidence of sensitization of the denervated

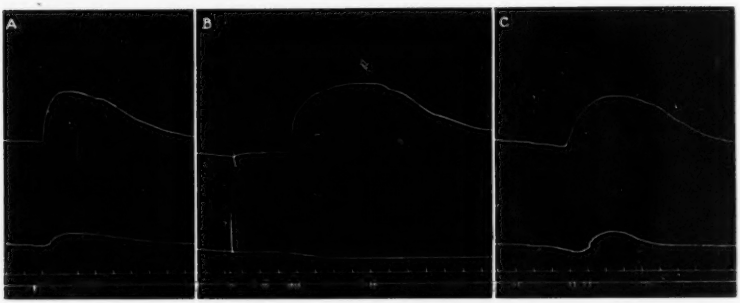


Fig. 4. Influence of period of local application of acetylcholine on the responses of the membrane. Adrenals ligated; eyeballs removed. Upper record: right membrane (decentralized 11 days). Lower record: left membrane (decentralized acutely).

A. Intravenous injection of adrenaline (2$\gamma$).

B. The successive pairs of lower signals denote: application of cotton pledgets soaked with acetylcholine (1 per cent) in Ringer, first to the right, then to the left superior cervical ganglion; removal of these pledgets (right, then left); washing with Ringer; further washing.

C. As in B.

ganglion, it was deemed desirable to attempt to separate further the ganglionic from the direct effects on the membrane. For this purpose animals, with the superior cervical ganglion chronically denervated, were prepared, in general description, as follows. The still intact cervical sympathetic was cut; the adrenals were ligated; the eyeballs were removed; the external carotids were tied immediately below the lingual arteries; and the common carotids, carefully cleared of loose tissue for about 3 cm. low in the neck, were enclosed in a loose loop of thread. When an injection was to be made the artery was gently lifted in the loop until it rested on a finger tip and the very fine needle of the syringe was pressed obliquely through the arterial wall into the lumen. Only rarely was there any

persistent bleeding when the needle was withdrawn, and no perceptible clot formed in the vessel.

The responses of the membrane to adequate doses of acetylcholine thus injected were then found to consist of two components: an initial sharp contraction with a latency of about 1 sec. and a late slower, further contraction with a latency between 9 and 20 sec. Although the relative height of these two components varied with the dose of acetylcholine, the state of the ganglion and the different animals, the latencies were quite typical and allowed thus a ready differentiation.

Similar injections of acetylcholine, made after removal of the superior cervical ganglia, resulted in the complete disappearance of the initial sharp contraction and did not modify the delayed component. Injections of atropine, on the other hand, reduced only slightly the magnitude (but not the delay) of the initial component, while abolishing the delayed contraction. It is quite certain, therefore, that the initial component denotes the response of the ganglion, while the delayed response is due to the direct action of acetylcholine on the membrane.

Different doses of acetylcholine in Ringer were injected by varying either the volume of a given dilution, or the concentration with a constant volume. It was found that the changes in concentration were more significant for grading the responses than the changes in volume injected. The usual procedure, therefore, was to inject 0.2 cc. of various concentrations. The doses were $5\gamma$ and multiples of that amount.

In 4 normal animals—i.e., with both cervical sympathetics cut acutely—the sensitivity of the ganglia was tested by determining the threshold doses of acetylcholine. This threshold varied from 20 to $40\gamma$, but was the same on the two sides (table 1, cats 1 to 4). Similar thresholds were later found in the normal control ganglia of the 7 operated animals (table 1).

In the 7 operated animals the sensitivity of the denervated ganglia was consistently about 4 times greater than that of the normal control ganglia—i.e., the threshold concentration of acetylcholine was one-fourth as great (table 1). In all these animals intravenous injections of adrenaline which evoked responses of the denervated side equal to those caused by a given small dose of acetylcholine (e.g., $10\gamma$) invariably elicited also a clear response of the normal control membrane. Injections of atropine (1 mgm. per kgm.) depressed slightly the reactivity of the ganglia, i.e., the threshold was increased by about 30 per cent. The depression was similar on the two sides. The different sensitivity of the ganglia persisted, therefore, after atropine.

Figures 5 and 6 illustrate the results. In figure 5A is shown the action of adrenaline ($10\gamma$) injected intravenously in cat 10 (table 1). In B an injection of acetylcholine ($5\gamma$) into the right carotid (normal side) caused

only delayed contractions (10 sec., timed by stop watch) of both membranes. In C the same dose injected into the left carotid caused a prompt response (1 sec.) of the left ganglion (denervated 12 days); the less sensitive right membrane did not contract. In D is shown the prompt response (delay 1 sec.) of the normal right ganglion when 40γ were injected into the right carotid; the left membrane has again only a delayed (10 sec.) contraction, coincident with an increase of response in the right side; the simultaneous ordinates bring out the time relations of the two effects. In E, finally, is shown the disappearance of the prompt component when the same injection as in D was repeated after removal of the two superior cervical ganglia.

TABLE 1

*Threshold doses (γ in 0.2 cc. Ringer) of acetylcholine which elicited responses of normal and denervated ganglia*

Injections into the common carotid; external carotids tied

| | CAT NUMBER | DENERVATED GANGLIA | NORMAL GANGLIA | |
|---|---|---|---|---|
| Normal cats | 1 | | 40 | 40 |
| | 2 | | 30 | 30 |
| | 3 | | 40 | 40 |
| | 4 | | 20 | 20 |
| Operated cats | 5 | 10 | 40 | |
| | 6 | 5 | 20 | |
| | 7 | 10 | 40 | |
| | 8 | 5 | 20 | |
| | 9 | 5 | 20 | |
| | 10 | 5 | 30 | |
| | 11 | 10 | 40 | |

In figure 6 (cat 9, table 1) the influence of atropine is shown. At A is the prompt contraction (delay 1 sec.) of the left membrane (decentralized 14 days) upon injecting 5γ of acetylcholine into the left carotid artery before atropine; a similar injection into the right carotid did not produce any response. Between A and B atropine (1 mgm. per kgm.) was administered. The remainder of the figure shows the following events: B, injections of acetylcholine (10γ) first into the right, then into the left carotid; C, adrenaline (10γ) intravenously; D, acetylcholine (40γ) first into the right, then into the left carotid; E, removal of the superior cervical ganglia while the drum was standing still, with contractions of the membranes in response to the mechanical stimulation; F, acetylcholine (40γ) successively into both carotids; G, adrenaline (10γ) intravenously.

Injections of larger doses of acetylcholine (e.g., 40 to 80γ) resulted in approximately equal responses of both the decentralized and the control

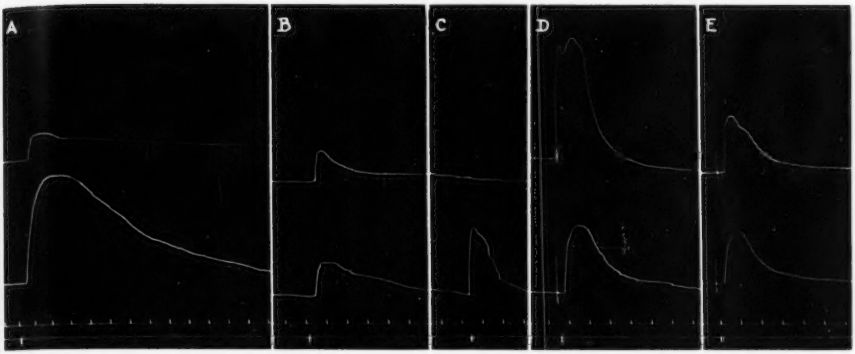


Fig. 5. Prompt and delayed responses to injections of acetylcholine into the carotid arteries. Upper record, right (normal); lower record, left (decentralized 12 days). For explanation see text.

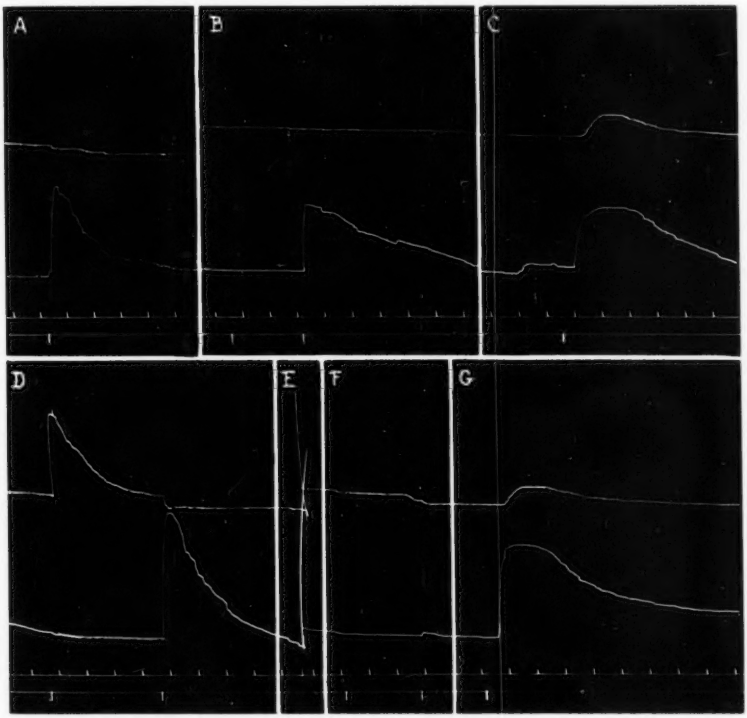


Fig. 6. Difference in sensitivity to acetylcholine between the normal and the denervated ganglia after atropine. Upper record, right (normal); lower record, left (decentralized 14 days). For explanation see text.

membranes. The differences in the paralytic effects of large doses of acetylcholine on the ganglia were not studied (cf. Brücke, *loc. cit.*).

Discussion. *Brücke's experiments.* Since Brücke's results and conclusions differ from those we previously reported and those we here report, it is pertinent to analyze his work to learn, if possible, the source of the discrepancies.

Brücke states that in some exceptional animals degeneration of the preganglionic fibers produced no sensization of the membrane to adrenaline; indeed, he even observed a desensitization—i.e., smaller responses on the denervated than on the innervated side. He does not mention how many animals were studied and what proportion showed this anomalous behavior. The preganglionically denervated membrane has been extensively studied in this laboratory (Hampel, 1934; Cannon and Rosenblueth, 1936; Simeone, 1937; Simeone, 1938). To the number of animals used in these various studies may be added the present series of 25 cats similarly prepared. In all instances in all these investigations a clear sensitization to adrenaline has developed after the delay pointed out by Hampel (1934).

Another discrepancy between Brücke's results and those here reported is found in the effects of removal of the eyeballs on the responses to adrenaline. Approximately equal contractions of the normal and the decentralized membranes, which Brücke found to be the rule, were in our experience the exception (fig. 2). The difference in the responses, which denotes sensitization, decreased, to be sure, after the procedure, but was still clearly measurable (figs. 3, 4, 5 and 6).

These two discrepancies are probably explained by the fact that Brücke usually employed unnecessarily large doses of adrenaline (30 to 70$\gamma$ in his figs. 1 to 5), as compared with the more moderate doses which we have employed (less than 20$\gamma$). The difference between the maximal responses of normal membranes and those sensitized either by denervation or by cocaine is negligible. The sensitization is only apparent in the submaximal range. Indeed, Brücke's one published record (his fig. 6) in which he used less than 30$\gamma$ of adrenaline reveals sensitization of the denervated side.

It is not surprising that Brücke's results should differ from ours, for two reasons. First, he did not duplicate our experimental conditions. In our experiments on intravenous injections of acetylcholine we used curare, which he did not employ. It was shown, in sections II and III, that curare modifies significantly the results. In our experiments on local application of acetylcholine to the ganglia we avoided the use of any drug other than the anesthetic. Brücke, on the other hand, declares that he routinely atropinized the animals in all experiments. The second reason for his different results is that, as was mentioned above for adrenaline, he used mainly excessive doses of the substances tested. To duplicate, approximately, the effects of large doses of adrenaline, he had to use also large

doses of acetylcholine (2 to 5 mgm. intravenously in his fig. 4, as contrasted with the doses of less than 1 mgm. employed by Cannon and Rosenblueth).

The last criticism does not apply to Brücke's local application experiments. It is, however, impossible to comment on them because he does not publish any time-scale for his records, nor does he state whether the latency was similar on the two sides, nor, finally, does he tell how long he applied the acetylcholine. It was shown in section IV (fig. 4) that the period of application may modify the results.

Brücke's intra-arterial injection experiments differ from those reported here in that he injected into the right subclavian artery, instead of the carotids. His injections may not have been uniformly distributed to the two ganglia. Furthermore, he did not ligate the external carotids, and he used as an anticoagulant chlorazol fast pink, a substance whose effects on the ganglia are unknown. We fail to find in these, as in the other experiments, any criterion or any controls by which Brücke separated the action of acetylcholine on the ganglion from the direct effects on the membrane.

*The sensitization of the membrane by preganglionic denervation.* Our results (section I) confirm Elliott's (1905) conclusion that the membrane is sensitized to adrenaline by preganglionic denervation. All the smooth muscle in the orbit appears to be affected thus, and not merely some of it, as Brücke's results might suggest.

The different effects of removal of the eyeballs on the two sides, increasing the responses of the normal membrane and decreasing those of the decentralized membrane (fig. 2), is at present inexplicable. A more detailed knowledge of the mechanical conditions which prevail when the smooth muscle in the orbit contracts appears to be necessary for an explanation of this puzzling paradox.

The membrane is rendered more sensitive by preganglionic denervation not only to adrenaline, but also to acetylcholine (section II). Furthermore, the opposite influences of atropine and curare on the responses to acetylcholine (fig. 3) suggest that the sensitivity to these drugs is likewise affected by the operation.

*The sensitization of the ganglion by denervation.* It is important at this stage of the discussion to state explicitly what we mean by "sensitization." In this and in previous work, when we have said that a tissue was rendered more "sensitive" than normally to a given substance by a given experimental procedure or operation, we meant that the doses of the substance sufficient to evoke a given submaximal response after the procedure or operation were smaller than before. The important qualification may be stressed that the responses should be submaximal, preferably only liminal. As pointed out before, the maximal contractions of the denervated and the control innervated membrane are practically equal. A large dose of

adrenaline may evoke maximal effects on the two sides, and thus reveal no sensitization, whereas a small dose of the hormone may cause very different effects on the two sides—for instance, 60 per cent of maximal on the denervated membrane and only 5 per cent on the control.

According to this criterion for sensitization, the data here reported are conclusive evidence that the denervated ganglion is more sensitive to acetylcholine than the normal. Brücke (*loc. cit*). showed that paralysis produced by large doses of acetylcholine occurs for lower concentrations on the denervated than on the normal side. It should be expected, therefore, that with large doses of the substance the contractions of the chronically decentralized membrane may be even smaller than those of the normal side. Some of Brücke's results support this inference.

The effects of atropine and curare (section III) suggest that the susceptibility of the ganglion to these drugs is likewise modified by denervation. For this reason the evidence obtained from intravenous injections of acetylcholine, after treatment with these drugs, cannot be readily evaluated. The results obtained by applying directly to the ganglia pledgets wet with acetylcholine (section IV) are not open to this criticism. When the pledgets had, however, peripheral direct effects on the membrane, these effects could not be immediately separated from the stimulating action at the ganglion. The experiments in which acetylcholine was injected into the common carotids after the external branch was tied (figs. 5 and 6) circumvent both criticisms.

The results detailed above do not elucidate the manner of sensitization of the denervated ganglion to acetylcholine. It may be partly due to disappearance of a large proportion of the normal cholinesterase (Brücke, 1937). The differential sensitivity to potassium ions, however, reported by Cannon and Rosenblueth (not confirmed by Brücke, 1938), would suggest that the decrease of cholinesterase will not account for all the changes which take place after denervation.

Simeone, Cannon and Rosenblueth (1938) reported that partial denervation of a superior cervical ganglion—i.e., degeneration of some of the preganglionic fibers connecting with it—renders the ganglion more sensitive to the nerve impulses delivered by the remaining preganglionic fibers. Stimulation of these remaining fibers evokes greater ganglionic responses than normally. Such sensitization of partially denervated ganglion cells to nerve impulses would constitute a serious objection to the theory of chemical synaptic transmission by acetylcholine if the neurones were not likewise hypersensitive to acetylcholine after denervation. Since the hypersensitivity does occur, the results of Simeone, Cannon and Rosenblueth not only are not an objection to the chemical theory of ganglionic transmission, but actually furnish an additional argument in its favor.

SUMMARY

The effect of denervation of the superior cervical ganglion upon its sensitivity to acethycholine was studied in cats. Three methods were employed. The first two, intravenous injections of acetylcholine after atropine and curare, and local applications of acetylcholine solutions to the ganglia without any drugs (fig. 4), merely confirmed previous observations of Cannon and Rosenblueth (1936). These observations have been contested by Brücke (1938).

In the third method acetylcholine was injected into the common carotids after ligation of the external carotids. This method allows a ready differentiation of the effects of acetylcholine on the ganglion from the direct effects on the nictitating membrane, the indicator of ganglionic discharge (figs. 5 and 6). The denervated ganglia were consistently about 4 times more sensitive than the normal control ganglia, as measured by threshold doses (table 1).

The problem of the sensitization of the nictitating membrane to adrenaline after preganglionic denervation was also studied (figs. 1 and 2). The sensitivity of the membrane to both adrenaline and acetylcholine is increased by chronic decentralization (fig. 3); its sensitivity to atropine and curare is probably also modified thereby (fig. 3, p. 280).

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# SOME CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF THE GONADOTROPIC ANTAGONIST[1]

CARL A. BUNDE AND ARTHUR A. HELLBAUM

*From the Department of Physiology of the University of Oklahoma School of Medicine, Oklahoma City*

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The presence of a substance in the hypophysis which, when injected intraperitoneally, antagonized the effects of gonad stimulators, was evident in some of the earliest experiments with extracts of hypophyses. In 1927 Smith (14) noticed in hypophysectomized rats that the gonadal repair of pituitary transplants was prevented by intraperitoneal injections of hypophyseal extracts. The repair of the thyroid and adrenal cortex was not inhibited, thus indicating that the inhibition was specific for gonadotropic hormones.

The first attempt to identify this substance, which Evans et al. (4) have termed antagonist, was made by Evans and Simpson (5). They were able to make a partial separation of growth hormone from the gonadotropic hormone and found that the growth fraction contained the antagonist. Leonard (9) found the antagonist in Van Dyke's growth preparation. However, later Leonard (10) found that the most potent growth preparation contained no antagonist but it was found in a less potent growth fraction which also contained gonadotropic activity. This association of antagonist with the gonadotropic principle agreed with Evans, Simpson and Austin (6). They found the synergist (follicle stimulator) soluble and the antagonist insoluble at pH 4.4, but the luteinizing factor could not be eliminated from consideration since it too is insoluble at pH 4.4. In 1936 Evans and co-workers (4) published a method of preparing antagonist, but no data were given on results of subcutaneous injections of an in vitro mixture of their antagonist preparation and follicle-stimulating hormone in order to establish the presence or absence of the luteinizing factor. Furthermore their preparation produced large, heavily-luteinized ovaries in adult female rats and resulted in large, luteinized ovaries and prolonged gestation in pregnant rats. This latter effect has been found to be a result of gonadotropic action (2).

In this report the antagonist factor has been prepared free of gonado-

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tropic hormones by subjecting sheep anterior pituitary preparations to boiling and different pH levels. The use of the antagonist, thus prepared, permits a further study of its chemical and physiological nature.

PROCEDURE. Immature female albino rats 21 days old were used for all tests of follicle-stimulating and luteinizing hormones, and 21 to 23 day old animals for antagonist and unfractionated gonadotropic preparations Injections were made in 0.5 cc. amounts twice daily for 3 days and autopsy performed 120 hours after injections were started. The antagonist was assayed by intraperitoneal injections of the material to be tested, concurrent with subcutaneous injections of constant amounts of unfractionated gonadotropic extracts.

Boiling of extracts was done by placing vials containing the preparations in a water bath. Determinations of pH were made by means of a Beckman glass electrode pH meter. All preparations were adjusted to a pH of 7.0 before injections were made. Extracts were kept at a temperature of 14°C. during the time they were at either low or high pH. All extracts used were prepared in this laboratory according to a slight modification of the method described by Fevold and Hisaw (7).

The data are arranged in two tables depending upon the extract used as a source of antagonist. In table 1 an unfractionated sheep pituitary extract (Un.B) was used as a source of antagonist. This extract after each type of treatment was tested for antagonist, follicle stimulator, and luteinizer. Luteinizing fractions of sheep pituitaries (L.H.A and L.H.B) were used as the source of antagonist in table 2. After treatment, the L.H. fractions were tested for antagonist and luteinizer. The L.H. preparations contained no follicle-stimulating activity.

RESULTS. As shown in table 1, 0.2 gram equivalent of unfractionated sheep pituitary extract (Un.B), injected subcutaneously, produced ovaries averaging 50.5 mgm., but when intraperitoneal injections of 0.3 gram of the same preparation were made simultaneously with the subcutaneous injections, the ovaries averaged only 22.7 mgm. Using these amounts as standards for comparison, the extract Un.B was boiled for 15 minutes which did not completely destroy the gonadotropic activity but at the longer periods of 25, 60 and 120 minutes no ovarian stimulation was obtained even with as much as 0.3 gram equivalent. Boiling also destroyed the luteinizing hormone, for after boiling 0.3 gram Un.B for 60 minutes and testing with a purified follicle stimulator, no luteinization or augmentation occurred. *However, boiling, even for two hours, destroys none of the antagonist.*

When Un.B is subjected to a pH of 11.2 for 120 hours, neither the antagonist nor the gonadotropic activity is affected, but a pH of 13.0 for 72 hours destroys antagonist as well as all gonadotropic hormones. Therefore no separation can be made by subjecting the mixture to high pH.

A pH of 1.0 for 72 hours did not decrease the amount of antagonist in 0.3 gram Un.B but destroyed the follicle-stimulating activity. Apparently

TABLE 1

*The effect of boiling and pH changes on antagonist and gonadotropic hormones present in unfractionated anterior pituitary extracts*

| NO. OF RATS | AMOUNT, TYPE AND TREATMENT OF EXTRACT | | MEAN OVARY WEIGHT |
|---|---|---|---|
| | Subcutaneous injections | Intraperitoneal injections | |
| | | | *mgm.* |
| 18 | 0.2 gm. Un.B† | | 50.5 |
| 3 | 0.3 gm. Un.B—boil 15 min. | | 18 |
| 3 | 0.3 gm. Un.B—boil 25 min. | | 12.5‖ |
| 6 | 0.3 gm. Un.B—boil 60 min. | | 11.5‖ |
| 3 | 0.3 gm. Un.B—boil 120 min. | | 13‖ |
| 14 | 0.2 gm. Un.B | 0.3 gm. Un.B | 22.7 |
| 4 | 0.2 gm. Un.B | 0.3 gm. Un.B—boil 15 min. | 22 |
| 3 | 0.2 gm. Un.B | 0.3 gm. Un.B—boil 25 min. | 18 |
| 3 | 0.2 gm. Un.B | 0.3 gm. Un.B—boil 60 min. | 26 |
| 3 | 0.2 gm. Un.B | 0.3 gm. Un.B—boil 120 min. | 20 |
| 7 | 1 gm. F.S.B | | 24.2§ |
| 4 | 1 gm. F.S.B*<br>0.3 gm. Un.B—boil 60 min. | | 19.5§ |
| 3 | 0.3 gm. Un.B—pH 10.8 for 48 hrs. | | 58 |
| 3 | 0.3 gm. Un.B—pH 11.2 for 120 hrs. | | 49.5 |
| 3 | 0.3 gm. Un.B—pH 13 for 72 hrs. | | 12‖ |
| 3 | 0.2 gm. Un.B | 0.3 gm. Un.B—pH 10.8 for 48 hrs. | 26 |
| 3 | 0.2 gm. Un.B | 0.3 gm. Un.B—pH 11.2 for 120 hrs. | 28 |
| 3 | 0.2 gm. Un.B | 0.3 gm. Un.B—pH 13 for 72 hrs. | 46 |
| 3 | 1 gm. F.S.B*<br>0.3 gm. Un.B—pH 13 for 72 hrs. | | 26.3‡ |
| 4 | 0.3 gm. Un.B—pH 1 for 72 hrs. | | 12.4‖ |
| 4 | 0.2 gm. Un.B | 0.3 gm. Un.B—pH 1 for 72 hrs. | 22.5 |
| 6 | 1 gm. F.S.B*<br>0.3 gm. Un.B—pH 1 for 72 hrs. | | 30.8¶ |
| 5 | 0.2 gm. Un.B | Tannic acid precip. of 0.2 gm. Un.B | 21.5 |
| 5 | 0.2 gm. Un.B | Supernatant of 0.2 gm. Un.B after tannic acid precip. | 40 |

* Extracts mixed in vitro.

† Gram equivalents of desiccated pituitary powder.

Un. = Unfractionated gonadotropic pituitary extract. F.S. = Follicle-stimulating fraction.

‡ = all follicles.

§ = slight luteinization in ovaries of one rat only.

¶ = corpora lutea in ovaries of all rats.

‖ = no stimulation.

traces of the luteinizer remained after this low pH treatment, as its addition to a highly purified pituitary follicle-stimulator resulted in slight augmenta-

tion and luteinization. The data on effects of pH on Un.B are presented in table 1.

The extracts of series B were prepared from the same pituitary powder. One half of the unfractionated extract Un.B was used without further

TABLE 2

*The effect of boiling and pH changes on antagonist and luteinizer present in the luteinizing fraction of anterior pituitary extracts*

| NO. OF RATS | AMOUNT, TYPE AND TREATMENT OF EXTRACT | | MEAN OVARY WEIGHT |
|---|---|---|---|
| | Subcutaneous injections | Intraperitoneal injections | |
| | | | *mgm.* |
| 6 | 0.25 gm. Un.A† | | 45 |
| 5 | 0.25 gm. Un.A | 0.4 gm. L.H.A | 21 |
| 5 | 0.25 gm. Un.A | 0.4 gm. L.H.A—boil 15 min. | 19.6 |
| 4 | 1 gm. F.S.A | | 28.5§ |
| 3 | 1 gm. F.S.A<br>0.4 gm. L.H.A* | | 41¶ |
| 5 | 1 gm. F.S.A<br>0.4 gm. L.H.A*—boil 15 min. | | 28§ |
| 18 | 0.2 gm. Un.B | | 50.5 |
| 4 | 0.2 gm. Un.B | 0.33 gm. L.H.B | 22.5 |
| 3 | 0.2 gm. Un.B | 0.33 gm. L.H.B—boil 30 min. | 24.3 |
| 4 | 0.2 gm. Un.B | 0.33 gm. L.H.B—pH 13 for 72 hrs. | 40 |
| 4 | 0.2 gm. Un.B | 0.33 gm. L.H.B—pH 1 for 72 hrs. | 18.6 |
| 7 | 1 gm. F.S.B | | 24.2§ |
| 6 | 1 gm. F.S.B<br>0.33 gm. L.H.B* | | 50¶ |
| 3 | 1 gm. F.S.B<br>0.33 gm. L.H.B* –boil 30 min. | | 24.6‡ |
| 5 | 1 gm. F.S.B<br>0.33 gm. L.H.B*—pH 13 for 72 hrs. | | 30.2‡ |
| 5 | 1 gm. F.S.B<br>0.33 gm. L.H.B*—pH 1 for 72 hrs. | | 24.8§ |
| 6 | 0.2 gm. Un.B | 0.33 gm. L.H.B – boil 1 hr. at pH 2.5 | 26.8 |

* Extracts mixed in vitro.

† Gram equivalents of desiccated pituitary powder.

Un. = Unfractionated gonadotropic pituitary extract. L.H. = Luteinizing fraction. F.S. = Follicle-stimulating fraction.

‡ = all follicles.

§ = slight luteinization in ovaries of one rat only.

¶ = corpora lutea in ovaries of all rats.

treatment and the other half was fractionated into luteinizer (L.H.B) and follicle stimulator (F.S.B). Since, as shown in table 2, 0.33 gram equivalent of L.H.B contained as much antagonist as 0.3 gram Un.B, and since we were unable to inhibit gonadotropics by intraperitoneal injections

of F.S.B, it was evident that all the antagonist went into the L.H.B fraction. Therefore, in subsequent work, luteinizing fractions of the pituitary were employed as sources of antagonist.

As seen in table 2, two luteinizing fractions were boiled, one for 15 minutes and one for 30 minutes, and, as in the case of Un.B, the luteinizer was destroyed but the antagonist was unaffected. L.H.B was also subjected to high and low pH with results similar to those shown in table 1 for Un.B except that all of the luteinizer was destroyed by a pH of 1.0 for 72 hours. A pH of 13 for 72 hours destroyed both luteinizer and antagonist.

A tannic acid precipitate of Un.B contained all the antagonist, none remaining in the supernatant fluid. These data are shown in the last two lines of table 1.

Significant inhibition was not obtained by subcutaneous injections of antagonist mixed in vitro with either follicle-stimulating hormone or unfractionated gonadotropic extracts.

The antagonist was not found in human pregnancy urine or pregnant mare serum, confirmining Leonard et al. (11) and Evans et al. (4). However, the gonad-stimulating effect of these substances is markedly decreased by the intraperitoneal injections of the pituitary antagonist.

Discussion. In order to establish the antagonist principle as a separate entity, it is necessary to eliminate the possible presence of all known anterior pituitary hormones. The experiments presented in this paper at once rule out both gonadotropics (F.S.H. and L.H.). Growth hormone could not be present after 2 hours' boiling (3); besides Leonard (10) did not find antagonist in a potent growth extract. The thyrotropic principle is water soluble at pH 4.2 (Greep, 8), and is thus in the F.S.H. fraction which contains no antagonist. Thyrotropic preparations are also heat-labile (8).

Riddle et al. (13) found prolactin to withstand boiling for 1 hour at a pH of 7.5 to 8.5 but loss of activity occurred when the extract was boiled at lower or higher pH. This was confirmed by McShan and French (12). In the present work, Un.B was boiled at a pH of 6.0, and L.H.B at a pH of 5.0, and in one group of animals at a pH of 2.5 (last line of table 2), with no loss of antagonist. McShan and French also report concerning prolactin, "gradual loss of potency in the presence of small amounts of acetic acid," but as shown in tables 1 and 2, a pH of 1.0 for 72 hours had no effect on the potency of antagonist. It therefore seems unlikely that the antagonist is prolactin. However, Bates et al. (2) have found that the stimulating effect of F.S.H. in mature fowls is inhibited by concurrent injections of prolactin. They conclude the action is due to a suppression of the animal's own pituitary. Antagonist must not act through the hypophysis since it neutralizes the injected gonadotropics in immature animals, as shown in this paper, and in hypophysectomized animals, as shown by Smith (14).

Evans and Simpson (5) claim to have destroyed the antagonist by heat but do not state the temperature or the time of heating. Our work shows antagonist to be unaffected by boiling for 2 hours.

We have found the antagonist to remain with the gonad-stimulating hormones in our regular extraction procedure for unfractionated extract. It is insoluble at pH 4.2 and passes with the luteinizing hormone through all subsequent treatment. Evans et al. (4) report that tannic acid renders ineffective the antagonist; however, we have found that antagonist is precipitated by tannic acid along with the gonadotropic hormones, and loses no potency by tannic acid treatment.

These data suggest the possibility of a procedure safely employing wide ranges of pH and temperature in the preparation of antagonist.

## SUMMARY

The pituitary antagonist inhibits the action of concurrent injections of gonadotropic hormone. It is present in unfractioned pituitary gonadotropic extracts, and upon fractionation it is found with the luteinizer.

Subjecting sheep anterior pituitary preparations to a high pH destroys the antagonist as well as the gonadotropic principles but extended boiling and a low pH destroy all gonadotropic activity without affecting the antagonist. The possibility of other known anterior-lobe hormones withstanding this treatment is discussed.

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# PLASMA PROTHROMBIN LEVELS IN VARIOUS VERTEBRATES[1]

E. D. WARNER, K. M. BRINKHOUS AND H. P. SMITH

*From the Department of Pathology, State University of Iowa, Iowa City, and the Marine Biological Laboratory, Woods Hole, Mass.*

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In all higher forms of animals the fibrin clot is an important element in hemostasis. In certain lower forms, fibrin clotting is a rudimentary process, or is entirely lacking. In certain arthropods, for example, hemostasis is accomplished largely by agglutination of circulating cells (5), somewhat similar to the platelet thrombus formation seen in mammals. In the present paper we shall show that the thrombin-fibrin clotting mechanism has a gradual evolution up through the various forms of vertebrates. The plama prothrombin level in the dogfish, for example, is only 8 per cent of that found in many mammals.

METHODS. Our technique for the titration of prothrombin, previously described (6, 8), has recently been modified in several particulars. We have found that the fibrinogen solution is far more stable if all steps in its preparation are carried out in a room maintained at 5°C. The centrifuge, mounted in this room, is of the angle type, thus minimizing heating due to air friction. The ammonium sulphate solution, saturated at this temperature (about 35 per cent) contains a trace of potassium oxalate to insure against the action of any calcium salts which may be present as impurities in the various reagents. At 5°, fibrinogen is less soluble in ammonium sulphate mixtures than at room temperature. To effect the precipitation of fibrinogen about 4 volumes of "adsorbed plasma" (7) are treated with 1 volume of the saturated ammonium sulphate solution. Slight variations in amount of the latter are employed if the precipitate appears to be unduly voluminous or unduly scanty.

In the more recent experiments the technique has also been modified by the introduction of serological pipettes of 0.1–5.0 cc. capacity, thus eliminating the drop method as a means of measuring the various reagents used in the titration procedure.

Thromboplastin, and other solutions which deteriorate readily, are now

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preserved in a special storage box maintained at a temperature of −35° by means of an electrically-driven cooling unit. This obviates the need of using "dry ice," as reported in our earlier experiments. Thromboplastin preparations are made up in saline. In those prepared from lung and brain the organs were ground, mixed with an equal amount of saline and extracted at 5°C. 24 to 48 hours. They were cleared by centrifugalization, neutralized, and used in a dilution found for each particular plasma to give maximal yields of thrombin. Typically, the extract was diluted 5 to 24 times.

TABLE 1

*Plasma prothrombin levels in various species*

| SPECIES | NUMBER OF DETERMINATIONS | THROMBOPLASTINS EMPLOYED* | PROTHROMBIN CONVERSION TIME† | AVERAGE PROTHROMBIN LEVELS (DOG = 100) | AVERAGE DEVIATION FROM MEAN |
|---|---|---|---|---|---|
| | | | *min.* | *per cent* | *per cent* |
| Dog | | 3, 4, 5 | 2 | (100) | |
| Albino rat | 7 | 1, 3, 5 | 2 | 95 | 8.1 |
| Cat | 9 | 1, 3, 5 | 2 | 91 | 5.3 |
| Albino rabbit | 5 | 1, 3, 5 | 2 | 89 | 4.2 |
| Human | 11 | 2, 3, 5 | 4 | 84 | 8.8 |
| Guinea pig | 7 | 1, 3, 5 | 4 | 53 | 4.4 |
| Chicken (white leghorn) | 10 | 2, 5 | 3 | 50 | 2.8 |
| Turtle (*Chrysemys picta*) | 4 | 1, 3 | 3 | 42 | 1.0 |
| Sea bass (*Centropristus striatus*) | 2 | 4 | 3 | 31 | 2.0 |
| Stingray (*Dasyatis centura*) | 2 | 4 | 3 | 27 | 0.5 |
| Dogfish (*Mustelus canis*) | 4 | 2, 4 | 3 | 8 | 1.8 |

* Thromboplastins: 1 = homologous lung extract; 2 = homologous brain extract; 3 = beef lung extract; 4 = dog lung extract; 5 = cephalin, 0.3 per cent. in saline.

† Prothrombin conversion times, column 4, were determined with optimal quantities of lung extract, or brain extract, both homologous and heterologous. The thrombin-forming mixtures were the standard prothrombin titration mixtures, each containing 1 unit of prothrombin per cubic centimeter.

In the titration, calcium chloride was used in about the concentration previously reported, but varying slightly in different species to give maximal yields of thrombin. In every case, normal dog plasma was run simultaneously as a control.

Results. In table 1 are shown the plasma prothrombin levels of the various species studied. All values are expressed in terms of dog plasma, in which species the prothrombin level is arbitrarily set at 100. As we have shown (6) dog plasma normally contains about 350 units prothrombin per cubic centimeter. With this fact in mind the reader can calculate the unit values in the other species, if this information is desired. In any given species the normal variation in the plasma prothrombin level is

minimal. In no case is the average deviation from the mean more than 10 per cent, and in many cases it is decidedly less than this. No doubt, much of this variation is due to errors in technique.

The plasma prothrombin levels of mammals are seen to be higher than those of lower forms, but even among mammals the species differences are considerable. Guinea-pig plasma is only about one-half as rich in prothrombin as is the plasma of the rat or dog. In human plasma, our analyses show an intermediate value of 84 per cent of the canine level. We cannot agree with the reports of Quick (3, 4) that human plasma is only 20 per cent as rich in prothrombin as is the plasma of the rabbit. Our experience, cited in table 1, shows the two to be practially identical. It now appears that the low prothrombin values obtained in man by Quick are due to errors inherent in his technique. His titration technique is a one-stage procedure—an accelerated clotting time—which does not differentiate between amount of thrombin and speed of thrombin formation. Our table shows that the conversion of prothrombin into thrombin is much slower in human plasma than in rabbit plasma. Evidently, Quick mistook sluggish conversion in human plasma for a deficient quantity of prothrombin. Our two-stage procedure avoids this error, for it includes a preliminary incubation period in which the prothrombin is converted completely into thrombin, followed by a clotting phase on the addition of fibrinogen. This two-stage procedure permits the elimination of the variable conversion speed, and leaves the prothrombin concentration as the sole variable to be measured.

The long prothrombin conversion time is, no doubt, an important contributory factor in rendering man so highly susceptible to a number of different hemorrhagic diseases. The only other mammal in which the prothrombin conversion time has been found to be long is the guinea pig. It is a curious fact that man and guinea pig are the two species which are susceptible to scurvy. An inefficient clotting mechanism, no doubt, aggravates the tendency to bleeding in scurvy, but it is difficult to see what connection could exist between a long prothrombin conversion and an inability to synthesize adequate amounts of vitamin C.

The prothrombin level in chicken plasma is lower than that of the mammals studied, but higher than that of the cold-blooded animals. The level in the turtle is very little higher than in the bony fishes. Among the elasmobranchs, the level in the stingray is higher than in the dogfish, where the level is only about 8 per cent of the value in the dog. It is of interest that the whole blood clotting time in the dogfish is 4–6 minutes, which is about the same as in the dog. It is evident that the dogfish makes maximal use of its scanty supply of prothrombin. One might anticipate that compensation for the deficiency would be effected by

means of shortened prothrombin conversion time, yet the table shows that such is not the case. It appears likely that the cells in shed blood may liberate thromboplastin more readily than in the mammals studied.

The question of whether thromboplastin is specific for each species is one of some importance, a point recently reviewed by Moore, Suntzeff and Loeb (2). In most cases we have found that organ extracts from one species are almost equally effective in converting the prothrombin of the other species studied. However, in the case of chicken plasma, we have been unable to obtain satisfactory prothrombin conversion when extracts of canine or beef lung were used as sources of thromboplastin. However, with chicken plasma, we obtained the same prothrombin titer with cephalin prepared from beef brain as with thromboplastin derived from the tissues of the chicken. It is not clear whether cases of apparent specificity of thromboplastin represent true differences in composition, or whether the differences obtained are due to interfering reactions, associated, perhaps, with the admixture of foreign proteins.

As a source of thromboplastin, many organs have been tested, but in our experience, thromboplastin prepared from brain or lung has been most active, and has given the highest thrombin titers. Cephalin, prepared by the method of Howell (1), is a satisfactory thromboplastin, though it does give slower conversion of prothrombin than do the extracts of lung; yet if given time the same titer of thrombin is developed. We have already suggested (7) that the sluggish reaction of cephalin is associated with the fact that it is not as highly soluble as the thromboplastic material derived from lung. The chemical nature of the thromboplastin of the lung has not been established. It is possible that it is a cephalin-like substance which has been rendered more soluble by union with protein.

## SUMMARY

The plasma prothrombin levels are far higher in mammals than in the lower vertebrates. In dogfish plasma it is only 8 per cent as high as in plasma of the dog. In the plasma of bony fishes, reptiles, and fowls, the values rise progressively, broadly according to the position in the evolutionary scale. In human plasma the prothrombin level is slightly lower than in dog plasma, and in guinea-pig plasma the level is lower still.

Certain mammals, including man, show sluggish conversion of prothrombin into thrombin. Because of this sluggish conversion, these species are handicapped in the control of hemorrhage.

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# THE NATURE OF BLADDER RESPONSES FOLLOWING STIMULATION OF THE ANTERIOR HYPOTHALAMUS[1]

SHIH-CHUN WANG[2] AND FRANK HARRISON

*From the Institute of Neurology, Northwestern University Medical School, Chicago, Ill.*

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Bladder responses have been obtained following electrical stimulation of the brain stem (Karplus and Kreidl, 1909). The region of the forebrain and midbrain were carefully explored by Kabat, Magoun and Ranson (1936) in an attempt to locate the reactive points for contraction and inhibition of the bladder. Lichtenstern (1912) reported that no response to central stimulation was elicitable after severance of the sacral autonomic connections. Spiegel and Hunsicker (1936), in their studies of the conduction of cortical autonomic impulses, found that contraction and inhibition of the bladder did not disappear after section of both hypogastric nerves but ceased after additional division of the pelvic nerves. According to Beattie and Kerr (1936), the contraction of the bladder obtained from the stimulation of the anterior hypothalamus was abolished by atropinization. All the evidence seems to indicate that while most other responses obtained from the hypothalamic stimulation are sympathetic, the contraction of the bladder is a parasympathetic manifestation.

The present series of experiments was undertaken to clarify the nature of the bladder response following stimulation of the anterior hypothalamus and to study its modifications subsequent to injection of various sympathicolytic and parasympathicolytic drugs.

METHODS. Twenty cats were used in these experiments. Twenty milligrams of nembutal per kilogram of body weight, given intravenously, provided a satisfactory anesthesia. The urethra was exposed and cannulated with a small glass cannula, which was connected to a tambour recording system. Respiratory movements were recorded through a trachea cannula and tambour. Blood pressure was registered from the right carotid artery.

With the aid of the Horsley-Clarke instrument (Ranson, 1934), a bipolar nichrome-wire electrode, completely enamelled except at the tips, which were about 0.3 mm. apart, was introduced into the anterior hypo-

[1] Aided by a grant from the Rockefeller Foundation.

[2] Fellow of the China Medical Board.

thalamus; a reactive region was located, after which the electrode was left undisturbed. The stimulating current was provided by a Harvard inductorium having a dry cell (1.5 volt) in the pirmary circuit with secondary coil at 9 cm.

In each of twelve animals, both hypogastric nerves and the lumbosacral roots and corresponding segments of the cord were exposed and were sectioned one at a time to study the effects on bladder contraction following central stimulation. In the case of stimulation of the roots, no attempt was made to separate the ventral from the dorsal roots. On two occasions, the lower abdominal sympathetic chains and pelvic nerves were dissected for stimulation. In another group of eight cats, the effect of atropine and ergotamine on the central and peripheral bladder response was studied. The drug was administered through a hypodermic needle fixed in the great saphenous vein, and between injections a stylet was inserted in the needle.

After the experiment was completed, the roots were carefully identified. The point of stimulation in the brain was ascertained to be in the lateral hypothalamic area rostral to the infundibulum.

Results. In 2 animals, after several control contractions of the bladder were obtained by stimulation in the anterior hypothalamus, the roots from $L_7$ to $Coc_2$ inclusive were sectioned. A small but definite contraction or contraction followed by relaxation upon hypothalamic stimulation persisted. Similar results were obtained in three other animals in which the cord was transected at the level of $L_{6-7}$ (fig. 1b). Section of both hypogastric nerves abolished this residual response (fig. 1c). Stimulation of the peripheral cut-end of one of these nerves yielded similar bladder responses (fig. 1d). The possible influence of contractions of the abdominal muscles or respiratory movements was excluded by exteriorizing the bladder in some experiments and by the fact that the responses were absent following section of the hypogastric nerves. Sometimes, additional extirpation of the lower abdominal sympathetic chains was necessary to completely abolish the bladder response to central stimulation. Stimulation of the chain itself also occasionally yielded trivial contractile responses. Exclusion of the sympathetic innervation with the sacral nerves intact did not give an appreciable modification of the responsiveness. In such a preparation, if the sacral roots were then cut, the central bladder response (contraction) was totally abolished. In none of five experiments did stimulation of the hypogastric nerve give a pure inhibition of the bladder tonus.

Section of the last lumbar, the first sacral and the first two coccygeal roots on both sides did not influence the bladder tonus nor its response to hypothalamic stimulation. Furthermore, direct stimulation of these roots gave no bladder changes except that in one instance out of six experiments performed for this purpose stimulation of the first sacral roots

resulted in a feeble contraction of the bladder. Section of both third sacral roots would occasionally bring about a small reduction of the central bladder response, but direct stimulation of the roots themselves always caused a contraction of considerable size (fig. 2). Section of the second roots alone would impair the bladder response considerably and direct stimulation usually yielded a greater reaction than that obtained by stimulating $S_3$ (fig. 2).

The bladder of an animal in which the sacral roots were cut bilaterally or the cord transected at the level of the last lumbar segment showed no

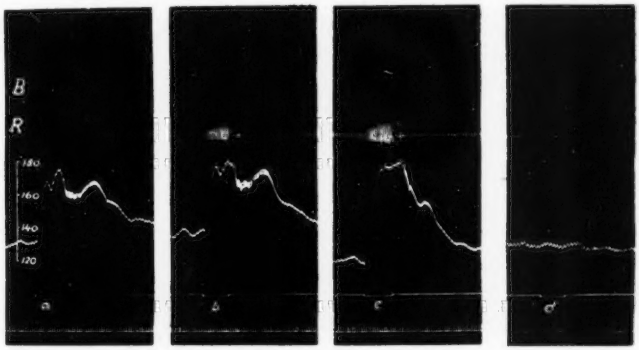


Fig. 1. The effect of transection of the spinal cord at the level of $L_{6-7}$ and severance of the hypogastric nerves on the contraction of the bladder (B) in response to anterior hypothalamic stimulation. Respiration (R) and right carotid blood pressure included to indicate the general reactive condition of the animal. Time in seconds.

a. Control. Stimulation of the anterior hypothalamus on the left side.

b. After transection of the spinal cord at $L_{6-7}$. Same point stimulated; a small but definite contraction of the bladder still present.

c. After additional section of both hypogastric nerves; bladder tonus slightly raised. Same point stimulated; bladder response abolished.

d. Stimulation of the peripheral cut-end of the right hypogastric nerve; similar contraction of the bladder as obtained in record b.

apparent change of its tonus, but spontaneous contractions, if present before the cut, were definitely reduced in magnitude and frequency of occurrence. In one experiment, the bladder showed unusual spontaneous peristaltic waves. On stimulation of the anterior hypothalamus, the bladder showed a prompt inhibition of the spontaneous motion and definite relaxation of the bladder tonus, which lasted about a minute after cessation of the stimulus; both disappeared after transection of the cord at the level of $L_7$. Subsequent to this, the sacral roots were stimulated and usual contraction of the bladder obtained.

With 3 mgm. ergotamine methansulphonate (Sandoz Chemical Works,

Inc., N. Y.) given intravenously, stimulation of the sacral roots remained effective and gave a contraction of normal height. The blood pressure showed a slight fall, which did not disappear after atropinization. Following administration of atropine, the bladder responded to pelvic nerve stimulation in the same prompt manner as before, but the tonus returned to normal earlier than usual (fig. 3). The initial contractile activity of the bladder, however, remained little changed even after as much a 50 mgm. atropine had been administered. A similar effect was obtained as regards its response to central stimulation, except that atropine prolonged its period of latency and somewhat lessened the contractile response (fig. 4).

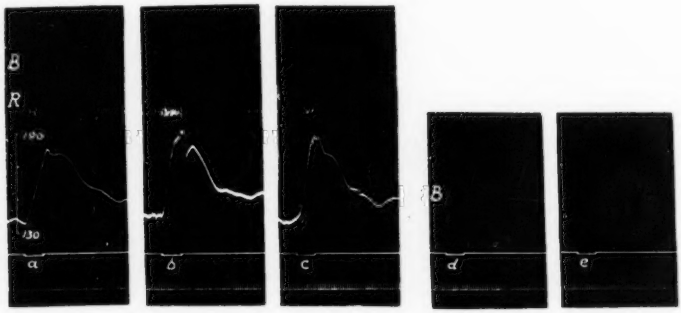


Fig. 2. The effect of section of both third sacral roots and then both second sacral roots on the central bladder response (B), and bladder responses to stimulation of those roots. Time in seconds.

a. Control, with both hypogastric nerves already cut. Anterior hypothalamus stimulated.

b. After section of the third sacral roots on both sides. Same point stimulated; slight reduction of contraction of the bladder obtained.

c. After section of the second sacral roots on both sides. No more contraction of the bladder on central stimulation.

d. and e. Responses of the bladder to stimulation of the second and third sacral roots on the right side.

On the other hand, the central bladder response was found to be very vulnerable to ergotamine administration. One milligram ergotamine methansulphonate, given intravenously, was sufficient to impair severely or abolish the central stimulating action on the bladder (fig. 5). After this dose of ergotamine, a primary fall of the blood pressure during the stimulus, succeeded by a rise which lasted for two minutes, was obtained instead of the prompt and transient pressor response formerly obtained on the hypothalamic stimulation.

Discussion. Contrary to the general belief that hypogastric nerves do not contain motor fibers to the fundus of the bladder, many investigators

working on cats (Sherrington, 1892; Langley and Anderson, 1896; Elliott, 1907) have observed a simple contraction of the bladder or contraction followed by inhibition of the detrusor urinae as a result of stimulation of the hypogastric nerve. Lately, it has been known that the sympathetic and parasympathetic nerves to the bladder contain both excitatory and inhibitory fibers (Griffiths, 1895; Macdonald and M'Crea, 1930; Gruber, 1933), and that the response of the bladder, a contraction or inhibition, is conditioned by muscle tonus and the nature of anesthesia (McCrea and Macdonald, 1934). This agrees well with the observation that, in a tonic bladder an occasional relaxation is obtained from stimulation of the anterior hypothalamus (Kabat, Magoun and Ranson, 1936). Its dis-

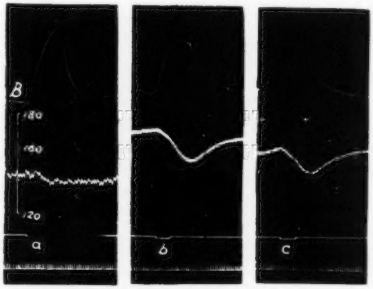


Fig. 3. The effect of ergotamine and atropine on the contraction of the bladder (B) following sacral root stimulation. Time in seconds.

a. Control. The right second sacral root stimulated.

b. After 3 mgm. of ergotamine methansulphonate I.V. (about 1 mgm. per kilo). Same root stimulated; bladder response well sustained and fall of the arterial blood pressure during stimulus.

c. After 10 mgm. of atropine I.V. (about 3.4 mgm. per kilo). Same root stimulated; bladder contracted to the same extent but tonus returned to normal earlier than usual. Same depressor response obtained.

appearance after transection at the level of the lower lumbar segments indicates that the impulse is transmitted mainly through the sacral autonomic system; and the inhibition is not to be regarded as a pure sympathetic manifestation. Since peripheral sacral root stimulation seldom yields an inhibition of the tonus of the bladder (McCrea and Macdonald, 1934), the central inhibitory responses occasionally observed are not completely explained by the high-tonic state. Nevertheless, the occurrence of a central inhibitory response is closely associated with a highly tonic bladder.

The small contraction occasionally followed by inhibition of the bladder in response to central stimulation after section of the sacral roots or transection of the cord at the level of $L_7$ is much like that following stimulation of the hypogastric nerve, and it promptly disappears after the severance

of these nerves (fig. 1). It is, therefore, reasonable to assume that the bladder impulse is normally brought down from the higher centers by both the sacral autonomic and the sympathetic systems and the contraction of the bladder must then be a resultant effect. Undoubtedly, the

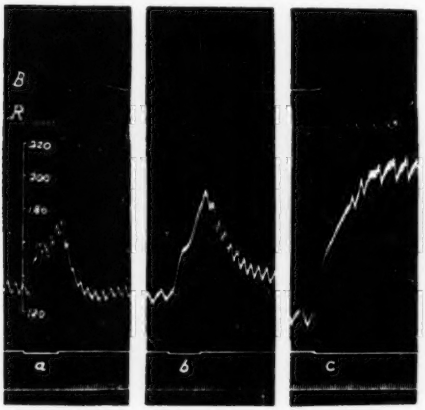


Fig. 4

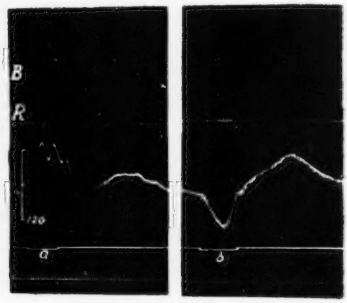


Fig. 5

Fig. 4. The effect of atropine on the central bladder response (B). Time in seconds.

a. Control. Anterior hypothalamus stimulated.

b. After 5 mgm. of atropine I.V. (about 2 mgm. per kilo). Same point stimulated; the latent period of the bladder response a little delayed and the contractile response slightly reduced. Blood pressure effect slightly augmented.

c. After another 25 mgm. of atropine I.V. (total about 12 mgm. per kilo). Same point stimulated for a longer period; further reduction shown, but the bladder remained moderately responsive to central stimulation.

Fig. 5. The effect of ergotamine on the central bladder response (B). Time in seconds.

a. Control. Anterior hypothalamus stimulated.

b. After 1.5 mgm. of ergotamine methansulphonate (about 0.5 mgm. per kilo). Very little contraction of the bladder on central stimulation. A fall of the blood pressure during the first half of the stimulus, followed by a rise lasting for two minutes.

former plays a much more important rôle in regulating the tonic condition of the bladder.

Opinion varies as regards the origin of the nerve fibers that constitute the pelvic nerve trunk (Gruber, 1933). In our series of six experiments, the relative importance of the sacral roots in the parasymapthetic innervation was studied by the influence of their severance on the central bladder response and also by the degree of the contraction of the bladder following

stimulation of each sacral root. And there remains little doubt that the second and the third sacral roots supply the great majority, if not all, of the pelvic innervation to the bladder. Perhaps, the second sacral roots give a few more fibers than the third, and rarely does the stimulation of the first sacral root give a positive bladder response.

The contraction of the bladder resulting from stimulation of the sacral roots is known to be resistant to atropinization (Edmunds and Roth, 1920; Cushny, 1910). With intravenous administration of 5 mgm. atropine, we have observed a short-lived contractile response to root stimulation which fails to last throughout the stimulus. Henderson (1923) and Henderson and Roepke (1934) observed the same phenomenon when they stimulated the pelvic nerve of an atropinized bladder or injected a dilute solution of acetylcholine intra-arterially. They postulated that atropine depresses the tonic mechanism which is mediated by the production of acetylcholine in the bladder, while the contractile response results from a different mechanism and is not depressed by atropine. However, as Langley and Anderson (1895) have observed, the contractile response of the bladder also shows considerable deterioration with larger doses of atropine. Contrary to Beattie and Kerr (1936), the bladder has responded to central stimulation in spite of atropine administration and its contractility remained after 30 mgm. of atropine.

The action of ergotamine (ergotoxine) on the nervous response of the bladder is even less well understood. It is generally supposed that ergotoxine reverses the contraction of the bladder produced by stimulation of the hypogastric nerve or injection of adrenaline (Dale, 1906; Elliott, 1907; Nelson and Calvery, 1938). In our experiment it is unlikely that the absence of the central bladder response under ergotamine (ergotoxine) can be explained by the neutralization effect of the reversed sympathetic response. Cushny (1910) found that ergotoxine reverses the contractile response to pilocarpine and advanced the idea that this parasympathetic drug stimulates the bladder via the sympathetic nerve endings. We have no evidence with which to explain the failure of the peripheral sympathicolytic property of ergotoxine to affect the central bladder response. Since the bladder remains reactive to pelvic stimulation, the myoneural junction of the parasympathetic system as well as the smooth musculatures of the bladder must be in at least fair condition. Therefore, the block is likely to be effected through a central mechanism. Brown and Dale (1935) found that ergotoxine raises the arterial blood pressure in decerebrated animals but lowers that of normal ones, and believed that this drug might have a central depressant action on the vasomotor centers in the forebrain. Then, there remains the possibility that ergotamine (ergotoxine) might depress the activity of various structures in the hypothalamus.

The effect of a change of blood pressure on stimulation of the anterior

hypothalamus following the administration of ergotamine is of some interest (fig. 5). Cannon and his collaborators obtained similar changes on stimulating the lower abdominal sympathetic chain following ergotoxine injection (Cannon and Rosenblueth, 1933; Rosenblueth and Cannon, 1933). They attributed the primary fall of pressure to stimulation of the surviving vasodilator fibers and the secondary rise to liberated sympathin E. Our experiments are not primarily designed for the study of ergotoxine action in this respect, and the experimental conditions are quite different from those of Cannon's. Yet, the similarity between these findings suggests that they might have the same mechanism, especially since sympathin is known to be liberated following stimulation of the hypothalamus (Magoun, Ranson and Hetherington, 1937).

## SUMMARY

Stimulation of the second and the third sacral roots as well as the hypogastric nerve yields a contraction of the bladder. The difference is only a matter of degree. The response of the bladder following stimulation of the anterior hypothalamus is manifested through the sacral autonomic as well as the hypogastric nerves.

The responses of the bladder to central and peripheral stimulation are resistant to atronization. The bladder so treated fails to maintain its usual sustained tonus during stimulus, but its power of contraction is retained and is reduced only following massive injection of atropine.

Ergotamine is potent in blocking the response of the bladder upon stimulation of the anterior hypothalamus. Its action is apparently not peripheral.

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# VASOCONSTRICTION IN THE HAND FROM A DEEP INSPIRATION

MICHAEL G. MULINOS AND ISRAEL SHULMAN

*From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University*

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The vascular status of the hand and lower forearm has been made the subject of the present study. This mass of tissue, approximately 700 cc. in volume, consists of muscle, skin, and bone. Any detailed study of the vascular condition of such an admixture of tissue as the forearm and hand must be capable of differentiating between the effects upon the vessels of the muscles and the effects upon the vessels of the skin.

It has been shown that following a deep breath, there is a shrinkage of the finger (7, 2) of the forearm and hand (18, 22) and of the hand and foot (24). In this article are analysed the data which have led to the conclusion that simple deep breathing results in constriction of the arterioles of the hand. Our observations with deep breathing are similar to those reported by Wright (1933) to result only from the inhalation of cigarette smoke.

EXPERIMENTAL. The vascular status of the hand, usually the right, was studied by five methods: 1, the pressure plethysmograph method of Hewlett and Zwaluwenburg (1909) which measures the rate of blood flow into the hand; 2, the simple plethysmograph which measures the volume changes in the blood present in the hand during an experiment; 3, the temperature of the skin; 4, the skin calorimeter as an indicator of the available heat of the skin; 5, the microscopic observation of the capillary tufts at the nail bed, which reveals the condition of the minute vessels and the behavior of the blood within them.

1. *The pressure plethysmograph method.* In the method of Hewlett (8), the 70 mm. Hg pressure which is applied to the forearm by means of a 5 cm. cuff is calculated to prevent the emptying of the veins until the pressure within them reaches that of the cuff (Lewis, 1924; Landis, 1930). During the venous obstruction, the hand within the plethysmograph swells at a rate proportionate to the rapidity of flow of blood into the hand. This conclusion is based upon the assumption that the cuff pressure does not significantly impede the arterial inflow (8). The tracings taken in the manner described (8) consist of 2 or more parts: the initial slope indicates rate of inflow and can be computed in cubic centimeters per

minute per 100 cc. of hand volume; and a secondary less steep slope which gradually becomes almost horizontal within 1 minute of the application of the pressure. A minimum of 2 minutes is allowed to elapse between readings because, although the rate of blood flow may return to normal within 1 minute, it requires 2 minutes for the skin temperature to return to normal following the release of the pressure (see p. 316). This delay makes the method discontinuous, a disadvantage that can be overcome by the performance of sufficiently numerous experiments to yield a composite picture.

The inclination of the inflow slope indicates rate of blood flow into the hand only if the blood pressure remains constant. Dr. Helen L. Taylor (23) proved that 1 or more deep breaths did not alter appreciably systemic blood pressure. Using the Tycos recording sphygmomanometer, never was she able to demonstrate a change in blood pressure of more than 5 to 7 mm. Hg (a value well within the experimental error) in from 1 to 10 determinations on each of 14 normal subjects.

Changes in the temperature of the air with the plethysmograph cannot alter the slope of the curve, because the plethysmograph interior is opened to the air during rest and the system is closed for no longer than 1 minute at any single determination. The moisture from sweating within the plethysmograph is minimized by desiccated calcium chloride.

When the subject has rested for from 15 to 30 minutes, and the pressure plethysmograph curves show good agreement in several records, the subject is instructed to take a deep breath. At the proper predetermined interval in the breathing cycle, the pressure is raised suddenly in the cuff and the volume recorder connected with the plethysmograph. The composite of many determinations demonstrates conclusively that the greatest reduction in blood inflow occurs at the height of the inspiration, when, indeed, it is usually reduced to zero. The change in rate of inflow varies somewhat from day to day in the same individual. There is much greater variation among individuals; some subjects have habitually only slight changes in inflow, while others usually stop completely at every deep inspiration. The subjects in the latter group also show greater inflow reactions to other procedures, such as inhalations of smelling salts, a painful pinch, drafts of air upon their person, or smoking.

In over 200 records obtained on 48 normal subjects (aged 20 to 50 years), the average blood flow into the hand was 8.5 cc. per 100 cc. of tissue per minute, minimum 3.5 cc., maximum 12.7 cc. Following a single deep inspiration it was decreased to 1.5 cc. or to 17 per cent of the original. In 32 of these 48 subjects there was complete stoppage and in 5 the change was slight. The slowing or stoppage lasted from 2 to 8 seconds. The vascular reaction to a deep breath is dependent upon an intact nerve supply to the limb (2); it occurs when the flow to the arm is mechanically

impeded (18) (p. 320). The reflex is not due to the cooling effect of the inspired air, because it is undiminished when the inspired air is warmed to body heat and saturated with moisture. Indeed, the slowing in the blood inflow rate may be obtained, though in diminished form, if the chest is put on tension forcibly but without allowing any air to enter the lungs. Bolton, Carmichael, and Sturup (2) believe that the reflex does not occur if the chest is mechanically prevented from stretching by strapping. By strapping the chest we have attempted to limit inspiration to the abdominal type, but we were unable to eliminate completely the obstructing effect of a deep breath on the peripheral hand-blood-flow. The Hewlett method does not show any cumulative effect on the rate of flow from successive deep breaths because 1 minute after the last of 10 deep breaths, the blood flow rate is usually the same as that after a single deep breath.

The inflow curve as obtained by the pressure method reaches a limit of growth within a minute of the application of pressure. Other factors remaining the same, the height reached by this curve is indicative of the tonus of the blood vessels at the moment. The greatest volume of the blood-bed is found in the capacity of the minute blood vessels as defined by Lewis (1927). Any change in the volume of blood in the hand is due to variation in the number that are patent, to alteration in the tonus of the minute blood vessels or to both.

*Summary.* The pressure plethysmograph method of Hewlett (8) indicates that in the majority of subjects, a deep inspiration is followed by complete stoppage of the blood inflow of the hand. This is due to a neurogenic stretch reflex (2).

2. *Volume changes with the plethysmograph.* Volume changes in the hand following a deep breath were measured with the simple plethysmograph. The method differs from that of Hewlett in that the pressure cuff is not used, and has the advantage of being continuous. Any changes in the temperature of the room air, or the air within the plethysmograph, will alter the volume curve and give false results. Changes in the vascularity of the hand within the plethysmograph, however, will result in alteration of temperature and air volume. These changes are part of the experimental technic and are unavoidable. In our experiment, the intraplethysmographic temperature was read at frequent intervals and the temperature effects were allowed for in the final analysis. Hewlett, Zwaluwenburg and Marshall (1911) and Freeman (1935) have attempted to obviate this source of error by filling the plethysmograph chamber with water kept at constant temperature. The objections to this method are that the skin of the hand becomes shriveled and macerated during a lengthy observation; and the hand is not permitted to maintain its own temperature, but must readjust itself to the new environment. To avoid these drawbacks, observations were made with air in the plethysmograph.

Changes in the temperature of the air resulting from alterations in the vascularity of the hand within the plethysmograph are usually slight as compared with the fall in skin temperature. Even total arrest of the circulation in the hand by an occluding pressure of 220 mm. Hg failed to alter materially the volume of the air in the plethysmograph during 5 minutes.

In figures 1 and 2 is shown the fall in hand volume resulting from a deep inspiration. The fall lasts almost 2 minutes, so that it cannot be due to the movement of the forearm incident to the deep breath which consumes less than 5 seconds. It cannot be due to shrinkage of the air within the plethysmograph from a cooling of the skin, because when the 2 phenomena

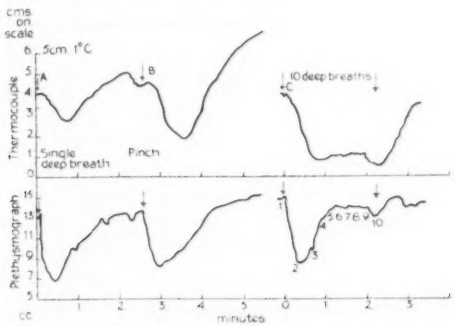


Fig. 1. Simultaneous records of finger skin temperature (skin calorimeter shown in fig. 4) and hand volume. At *A*, the subject took a single deep breath. At *B*, he was pinched on the arm on the side under experiment. At *C*, is shown the effect of 10 deep breaths, taken as indicated by the numbers. Note that the fall in hand volume precedes the fall in skin temperature.

are recorded simultaneously, the fall in volume *precedes* the fall in skin temperature (fig. 1).

The fall in hand volume following a deep breath varied in 25 healthy subjects from 1 cc. to 13 cc., with an average fall of 6.7 cc. The fall may be increased by simultaneous irritation as by a pinch (Capps, 1936) or by the inhalation of smelling salts. The hand volume returned to its previous level in from 1.5 to 2.0 minutes. The immediate cause of the fall in hand volume from a deep breath is to be found in a deficit between the outflow and inflow of blood. More blood leaves the hand than enters it. If a deep breath results in a constriction of the blood vessels of the hand on the arterial side of the capillary tuft, the constriction will register as a fall in hand volume. The volume should return to normal as soon as the constriction passes off. From the evidence of the pressure plethysmograph, and from capillary observation discussed on page 318, the fall in hand

volume outlasts the constriction by 30 to 60 seconds, figures 1 and 2. Constriction of the blood vessels on the arterial side of the capillary tuft would allow the pressure to fall in the vessels peripheral to the obstruction, in proportion to the degree and duration of the arterial obstruction. Upon release of the obstruction, the emptied capillaries and veins must be stretched again by the arterial blood in an interval of time indicated by the duration of the fall in volume as shown by the simple plethysmograph. This stretching may be hastened by increasing the venous and capillary pressure as in the Hewlett method.

The emptying of the minute vessels may be shown in another way. The volume of blood contained in the hand when the venous pressure is raised artificially to 70 mm. Hg depends upon the number and tonus of the patent blood vessels. If the venous outflow is obstructed at various stages

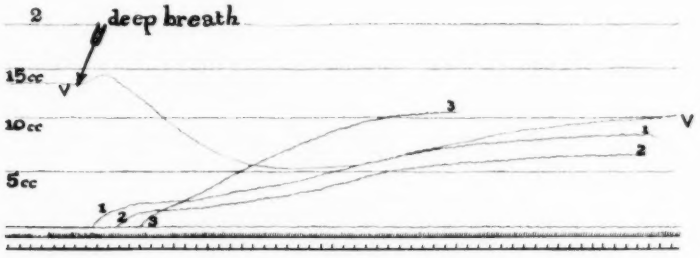


Fig. 2. A composite record of hand volume, *V*, and blood flow rates into the hand, curves *1*, *2*, and *3*. The lower time markings are in seconds. A deep breath was taken at the arrow for each curve, so that curve *1* was made at the height of inspiration; curve *2*, three seconds after a deep breath, and curve *3*, five seconds after a deep breath.

of hand volume loss following a deep breath, as shown in figure 2, graph *V*, it is possible to add to the resting-level blood-volume of the hand, that volume of blood which has been lost through the deep breath. The result is shown in curve *3* of figure 2. When curve *3* was made the hand had lost 4 cc. of blood incident to the deep breath. This curve is in consequence 4 cc. higher than any of the controls, at a time when its slope indicates that the rate of blood inflow has returned to normal. Changes in the pressure within the minute vessels may alter the capacity volume of the hand under any given venous pressure. Therefore, alterations in tonus alone cannot account for this "capacity volume" as has been claimed by Capps (1936).

Usually there was a more prolonged and a greater drop in hand volume from 10 deep breaths. The inhalations are taken at 10 second intervals from 1 expiration to the next inspiration, a total time of about 2.5 minutes.

Fifteen representative experiments were taken out of a group of over 100. The drop in volume varied from 5 cc. to 10.5 cc. with an average of 8.0 cc., as compared to 6.7 cc. for a single deep breath. The depression outlasted the last of the 10 deep breaths by from 1 to 3 minutes. Uhlenbruck (1924); Goetz (1935); Bolton, Carmichael, and Sturup (2), using the simple plethysmograph method, had previously noted a shrinkage in finger or hand volume from deep breathing.

*Summary.* It has been demonstrated by means of the simple plethysmograph that following 1 or more deep breaths there is a fall in hand volume which outlasts the last deep breath by 2 minutes. These results are in confirmation of those of the pressure plethysmograph discussed above and together demonstrate that the lower forearm and wrist as well as the fingers (7, 2) are concerned in the reflex.

3. *The fall in skin temperature after a deep breath.* The temperature of the skin of the fingers was determined by means of a thermopyle composed of 48 thermojunctions of silver-constantan, constructed after the method of Hill (1928) and Margaria (1930). The wire is mounted upon a bakelite frame about 4 x 1 x 1 cm. The constant or "cold" junctions are inserted into water in a thermos bottle and held in place by means of a rubber stopper. The wires are protected from corrosion by several coats of bakelite varnish. The variable junctions are covered by the finger of the subject during an actual experiment, and are thus protected from air currents. The poor heat conductivity of the bakelite frame allows the junctions to follow the temperature of the skin with great fidelity. The electromotive force developed by the thermopyle is recorded on a Micromax millivolt meter calibrated to read in degrees. This sensitive yet rugged instrument enabled us to obtain changes in finger skin temperature resulting from ten deep breaths taken at equal intervals during the course of 2.5 minutes. In 21 determinations on 18 normal subjects, the average drop in skin temperature was 0.78°C. with a minimum of 0.13° and a maximum of 2.27°C. This series of observations confirms the vasoconstrictor effect of deep breathing.

The drop in skin temperature from any procedure depends upon the approximate differential between skin and room temperatures (see Lewis, 1936). The smaller the differential, the less will be the drop, so that when the skin and room temperatures are equal, no drop from a constriction of the blood vessels is possible except from evaporation. In our experiments, the room temperature varied from 22° to 25°C., while the finger skin temperature was between 29° and 31°C.—a differential of 4° to 9°C. The maximum fall in temperature from vasoconstriction could not have exceeded 9°C. By obstructing the brachial artery for 5 minutes by a pressure of 250 mm. Hg to the arm, all blood flow to and from the hand and forearm is cut off, with a reduction in skin temperature similar

to that following 10 deep breaths. During the obstruction, skin temperature falls 1.76° to 2.48°C., confirming the findings of Lewis and Grant (1926). The average drop of skin temperature was 32 per cent of the room-skin temperature differential (table 1). The experimental spread of from 27 to 36 per cent shows fair consistency under the conditions of these experiments. In figure 3 there is plotted the fall in skin temperature from 5 minutes of mechanical obstruction together with the fall from 10 deep breaths. The diminution in skin flow from 10 deep breaths as measured in terms of skin temperature is equivalent to complete obstruction of blood flow for 2.5 minutes. The effects from the two procedures are not strictly comparable, however. The 10 deep breaths result in a fall in skin temperature due to vasoconstriction, plus a loss of blood from the skin; obstruction type of experiment traps the blood in the blood vessels so that the available heat in the skin of the hand is greater by about 15 cc.

TABLE 1

*Relation of skin temperature drop from occlusion (D) to the maximal possible drop (RT-ST)*

| SUBJECT | RT | ST | RT-ST = Δ | D DEGREES C DROP FROM OBSTRUCTION | $\frac{D}{\Delta} \times 100 =$ PER CENT |
|---|---|---|---|---|---|
| 1 | 24.0 | 31.92 | 7.92 | 2.48 | 31 |
| 2 | 26.5 | 31.73 | 5.23 | 1.89 | 36 |
| 3 | 24.0 | 30.54 | 6.54 | 2.24 | 34 |
| 4 | 25.0 | 30.70 | 5.70 | 1.76 | 31 |
| 5 | 24.7 | 30.37 | 5.67 | 1.54 | 27 |

RT = room temperature; ST = skin temperature.
All temperatures are in °C.

of blood. This difference in blood content may account for the fact that 2.5 minutes of intermittent obstruction from 10 deep breaths can cause a loss of heat from the skin of the finger equal to that from complete mechanical obstruction of blood flow for almost 3 minutes.

4. *The skin calorimeter shows loss of heat from a deep breath.* The thermopyle described above sacrifices speed of reactivity to the accurate measurement of skin temperatures. Quick changes in blood flow were recorded by means of a thermocouple as shown in the diagram of figure 4. A single chromel-copel thermojunction is soldered on a brass rod, the contact surface of which is 2 mm. in diameter. The rod expands to a diameter of 10 mm. and is connected to a brass cylinder 15 cm. long which dips into the water in a thermos bottle. The part of the rod outside the thermos bottle is jacketed in hard rubber. The "cold" junctions also dip into the water of the bottle but are electrically insulated from the water by being

coated with paraffin. The copper lead wires are connected to a suitable potentiometer and galvanometer as shown in figure 4.

The finger of the subject rests upon a hard rubber trough in the middle of which is the rod surface and the thermojunction. Heat from the skin of the finger is conducted along the rod into the thermos bottle, to be dissipated into the water. The electrical differential which is measured is a function of the skin temperature and the heat conducting efficiency of the brass rod. The greater the temperature differential between the skin and the water in the thermos bottle, the greater will be the electrical current

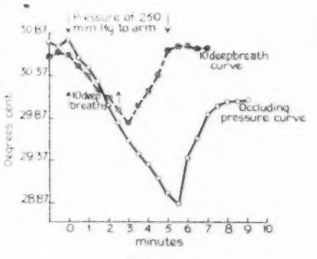


Fig. 3

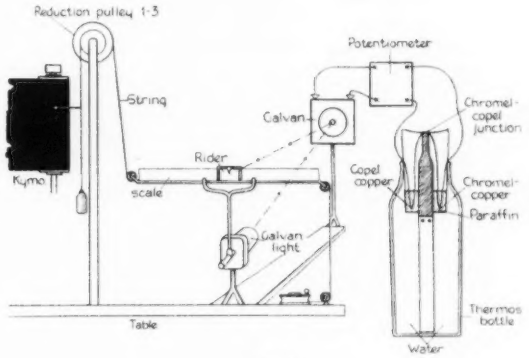


Fig. 4

**Fig. 3. Graph showing comparison between the drop in temperature from ten deep breaths, and complete occlusion of the brachial artery. The fall in temperature from the ten deep breaths, which consumed 2.5 minutes, was almost equivalent to the drop from 3 minutes of complete mechanical occlusion.**

**Fig. 4. The skin calorimeter. The e.m.f. developed by the differences in temperature between the two thermojunctions in the thermos bottle, and the variable junction of chromel-copel is recorded on the kymograph by means of the pulley and string arrangement. The kymograph record is reduced to one-third the scale reading. An assistant causes the rider to follow the galvanometer light during the course of an experiment.**

developed. The slightest change in the available heat of the skin will alter the temperature of the thermocouple of this skin calorimeter or thermostromuhr.

The water in the thermos bottle was allowed to come to room temperature and therefore remained constant throughout any period of observation (often as long as 2 hours). This apparatus proved so sensitive to minute changes in skin blood flow that when determinations were made with the hand exposed to the air currents of the room, the curves obtained were quite irregular and difficult to interpret. These disturbances due to local environment were obviated by incorporating the thermocouple in

the simple plethysmograph. In this way, not only was the hand guarded against drafts, but it became possible to measure simultaneously both the heat output of the skin of the finger and the volume of the hand as described in section 2. By means of this instrument, we were enabled to detect cooling of the skin of the fingers from a single deep breath.

Of 17 normal subjects, 15 or 88 per cent gave a definite drop in the heat output of the skin following a deep breath. In figure 1 is shown the remarkable fidelity with which the plethysmograph volume of the hand and the blood flow as measured by the skin calorimeter, change in the same direction.

*Summary.* By means of a thermopyle thermometer and a skin calorimeter, it was demonstrated that deep breathing (one to several deep inspirations), caused a fall in the skin temperature. The fact that the calorimeter readings showed considerable heat loss from a single deep breath is further suggestive evidence that there is vasoconstriction on the arterial side of the capillary tuft corroborating the evidence obtained with the pressure and simple plethysmographs.

5. *Microscopic observation of the nail fold capillaries.* The capillary tufts at the nail fold were observed through the Ultropack microscope attachment with a magnification of 11 x 15. During the observation, note was made of the length, width, and tortuosity of the tufts; of their number; and of the approximate rate of blood flow through the tufts. Three to 5 seconds following the application of a pressure of 70 mm. Hg to the forearm as in the Hewlett plethysmograph method, there appeared considerable slowing of the blood flow through the tufts. The slowing corresponded roughly with the change of slope of the rate curve of the pressure plethysmograph (p. 310). In an occasional subject, the flow of blood may cease momentarily. After the initial slowing of the blood flow, the stream of blood in the tufts moves forward in spurts which follow, in time, the pulse at the wrist. When the skin is cold, the pressure causes the capillary flow to become pulsating and arrested between pulse beats; while, when the skin is warm, the flow of blood continues slowly forward between the accelerating impulse of each beat. At no time is there any considerable stream of blood flowing through the tufts during the maintenance of the pressure. These observations would indicate that the venous pressure artificially raised to 70 mm. Hg is not sufficient to overcome the cuff obstruction, despite the higher pressure in the arteries. If, during the compression of the veins, the subject takes a deep breath, the forward pulsating movement of the blood in the tuft stops abruptly 2 to 3 seconds following the inspiration. The abruptness of the cessation of the flow indicates that the obstruction is very close to the capillary tuft. When the arterial inflow to the hand is arrested suddenly by a large pressure applied to the arm (see p. 319), microscopic observation of the tufts

shows that the arteries continue to empty for from 15 to 45 seconds. If the deep breath causes arterial rather than arteriolar constriction, the blood flow through the tufts would continue for a much longer time than is actually the case. Furthermore, since the pressure within the vessels of the skin on the venous side of the tuft is at least 70 mm. Hg, the veins must contract against the pressure in order to impede the flow of blood through the capillaries. Although this is possible, Lewis (1924) has shown that the minute vessels will not contract readily against such pressures, although they will resist much higher pressures when in a contracted state. Were the venous vessels to contract in response to a deep breath, it would be expected that some of the blood within them would be driven into the tuft in a retrograde fashion, a phenomenon which has not been observed to occur.

Apparently, the capillary tufts do not change in size or number during the 1 or 2 minutes that the pressure is applied. Any visible change in the diameter of the tufts, however, would, because of their prodigious number, represent a much greater change in the volume of the hand than any which we have ever obtained. There is at present no reliable method for the measurement of the small changes in calibre which may occur in the tuft under 70 mm. Hg pressure.

The flow of blood in the tufts of the normal nail fold is very rapid, appearing as a shimmer, the direction of which can be ascertained with ease. In 25 or 83 per cent of 30 normal subjects examined, a deep breath was followed by a complete standstill of the blood in the tufts lasting from 3 to 5 seconds. Some capillaries disappear entirely. We believe this to be due to the emptying of the tuft by plasma skimming and not to contraction, because the retreating column retains its shape while the entering column of blood has the same wide form as of the original. Were the capillary to reopen after a contraction the head of the entering column of blood would be narrow. The column of blood within the tuft accelerates rapidly following its arrest, confirming the findings with the pressure plethysmograph. The rate of blood flow within the tuft appears to have returned to the normal rate within 5 to 15 seconds after the deep breath.

The location of the contracting elements on the arterial side of the capillary tuft may be shown by the following experiment. When the circulation to the hand and fingers is interrupted by a pressure of 250 mm. Hg, movement of the blood in the capillary tuft under observation does not cease at once, but continues its onward course for from 15 to 45 seconds, slowly coming to a standstill. If, when all movement within the tuft has stopped, the subject takes a deep breath, there is a forward movement of the blood within the tuft synchronous with the height of inspiration, and a return to the previous status within 10 to 15 seconds.

The occurrence of arteriolar obstruction is well shown by observations

on 1 subject who gave a peculiar reaction to mechanical obstruction. A normal, healthy, male adult of 57 with blood pressure 130/86, presented normal capillary tufts under the microscope. Upon taking a deep breath, the blood stopped flowing through the tuft, but soon regained its normal rate. When a pressure of 250 mm. Hg was applied to the arm, the blood continued its forward course, usually for about 40 seconds. Five to 10 seconds later, however, the blood in the tuft began to move backwards, indicating that the arteries had overcontracted and were now relaxing. The retrograde movement, which was always slow, would last for from 15 to 70 seconds. If, during this retrograde movement, the subject took another deep breath, the backward movement stopped, and often reversed itself momentarily. We believe that this proves the deep breath vasoconstriction to be on the arterial side of the capillary tuft.

Discussion. The physiological significance of the vasoconstriction in the hand which follows a deep inspiration will not be considered. The discussion will be confined to the adequacy of the *proof* that such vasoconstriction does occur and to the determination of which vessels are involved. The occurrence of vasoconstriction from a deep inspiration is of significance because of the voluminous literature on the relationship between cigarette smoking and vasoconstriction and the bearing of the latter on such pathological states as thrombo-angiitis obliterans. Many authors (1, 3, 11, 12, 19, 20, 26, 27) have reported that cigarette smoking causes significant vasoconstriction only if inhaling is practised. Our results and those of Bolton, Carmichael, and Sturup (2) and Goetz (7) on the vasoconstricting effect of a deep breath show that the act of *inhaling* the smoke of a cigarette plays a major part in the ensuing vasoconstriction.

Of the 5 methods used in the present study, 3 (methods 3, 4, and 5) measure the blood flow through the skin of the fingers only, and 2 (methods 1 and 2) measure the total flow through the hand and lower forearm. In interpreting the results from the 2 types of measurement, it should be remembered that the methods measure separate but related phenomena, and that the plethysmographic methods include a greater portion of tissue than those which measure skin temperature alone.

The evidence that the shrinkage of the hand following a deep breath is due to vasoconstriction within the hand and not to humoral (hydrostatic) mechanisms is as follows: The vasoconstriction in a finger following a deep inspiration does not occur in patients whose sympathetic nerve supply to the hand has been previously destroyed (2). Lieb, Mulinos, and Taylor (18) demonstrated the independence from humoral mechanism in another way. The blood flow to the hand is stopped completely by the application of 250 mm. Hg pressure to the arm. While the capillary tufts at the nail bed are being observed, a deep breath usually causes an onward movement of the arrested blood in the tuft. These experiments prove 1, that the

vasoconstriction is neurogenic; 2, that it is independent of the systemic circulation, and 3, that the constriction is on the arterial side of the tuft.

The Hewlett pressure plethysmograph method indicates complete cessation of inflow for 2 to 8 seconds after a deep breath. To obtain this through humoral mechanisms alone, would necessitate a marked fall in systemic blood pressure. Wilkins et al. (1936) have lowered the blood pressure of patients by means of nitrites. They state that following sodium nitrite, "Two minutes before the fainting, the blood flow was still about 60 per cent of the original level, in spite of a pulse pressure of only 16 mm. Hg and a cardiac rate of 154 per minute. Thirty seconds before fainting, however, no blood flow or arterial pressure was obtainable" (p. 76). Our own studies show that blood pressure does not change during a deep breath and cannot account for the decreased blood flow rate.

Vasoconstriction of the hand is not due to cooling of the blood by the inspired air, for warming the inspired air to 37°C., and saturating it with moisture, does not diminish the constriction of the hand from a deep breath. Expansion of the chest is sufficient to bring about full constriction of the hand, for it occurs when thoracic breathing alone is practised (2). On the other hand, the constriction is less complete when thoracic expansion is limited voluntarily or by strapping.

## SUMMARY

1. By means of 5 methods the vasomotor status of the hand has been studied in normal human subjects. These methods are described in detail.

2. A deep inspiration causes marked vasoconstriction of the arterioles of the forearm and hand, but is especially marked in the skin of the fingers.

3. The vasoconstriction from a deep breath is due to a reflex and is independent of the blood flow and blood pressure in the hand and of the temperature and moisture content of the inspired air.

4. The constriction is exaggerated by any irritant or painful stimulus (pinch of skin, inhalation of smelling salts, tobacco smoke) which may accompany or shortly follow the deep breath.

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# ACUTE RIBOFLAVIN DEFICIENCY IN THE DOG[1]

HAROLD R. STREET[2] AND GEORGE R. COWGILL

*From the Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Connecticut*

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The water-soluble yellow-green pigment 6, 7-dimethy 9-(d-1 ribityl)-isoalloxazin, now called riboflavin (formerly lactoflavin), has been shown to be a member of the heat stable fraction of the vitamin B complex. Lack of this substance in the diet of growing rats leads to the failure of growth (7), loss of hair (11), cataract formation (5), and eventual death. It has also been shown that turkeys and chickens require riboflavin for growth and well-being (8).

Very little is known about riboflavin deficiency in the dog. However, since riboflavin appears to be universally present in living cells, (13) in most cases largely in non-diffusible form (presumably as a component of the "yellow enzyme"), it seems probable that all animals must either have riboflavin in the diet or be able to synthesize it. So far, only plants have been found to synthesize the pigment.

In the study of Zimmerman, Cowgill, and Fox (15), dogs were maintained on a purified diet containing adequate vitamin $B_1$, but lacking riboflavin and containing unknown amounts of the further heat stable vitamin B factors. It was found that these dogs suddenly collapsed in from 107 to 235 days on the experimental diet and died within a few hours unless treated with a potent vitamin preparation. In a few instances it was possible to save the animals by injection of liver extract. This was used because pure riboflavin was not available at the time.

Sebrell and Onstott (12) have recently found that dogs maintained on a purified diet lacking riboflavin exhibit just such a picture of sudden collapse, coma, and death. In several instances these investigators were able to save the animals after collapse by the injection of synthetic riboflavin in doses of from 2 to 5 mgm.

The present paper is a report of experiments in which dogs were maintained on a diet believed to contain adequate amounts of all dietary es-

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[2] Vitamin Research Fellow 1936–38; present address, Department of Physiology, Ohio State University, Columbus.

sentials required by the dog except riboflavin. The diet used here differed from that employed in the previous study (15) in that a tikitiki (rice polish extract) prepared by us was used as a source of the B complex vitamins other than riboflavin instead of the Block-Cowgill (1) $B_1$ concentrate used previously. Also, in the earlier study, Eli Lilly liver extract no. 343 was used as a source of riboflavin for treatment because it could be given parenterally, whereas pure riboflavin was administered in these later experiments.

We have fully confirmed the finding that animals on such a diet can be restored after the development of collapse by the prompt injection of riboflavin. Some observations have also been made of physiological changes in this deficiency state in an effort to determine the underlying cause of the sudden failure of the animals. In addition, information has been obtained on the amount of riboflavin required to maintain dogs in health.

EXPERIMENTAL PROCEDURE. The animals used for this study were adult mongrel dogs, ranging in weight from six to eight and one half kilograms at the beginning. All were given a vermifuge before being placed on the experiment.

*Diet employed.* The basal diet consisted of an artificial ration believed to contain all dietary essentials required by the dog except riboflavin. Its composition is shown in table 1. A record of the amount of food consumed daily by each animal was kept throughout the experiment.

Each control animal received in addition 25 micrograms of riboflavin[3] per kilogram of body weight daily. This was given in the form of a solution poured into the animal's mouth from a test tube.

In devising this diet, the most difficult problem that presented itself was the provision of adequate amounts of all the essential B vitamins other than riboflavin. Our solution consisted in the use of a particular tikitiki which we prepared from rice polishings. Tikitiki has been shown by Richardson and Hogan (11) and Day and co-workers (5) to contain all of the B vitamins required by the rat with the exception of riboflavin, which is completely lacking or present only in traces.

Our tikitiki was prepared by extracting rice polishings[4] with 25 per cent alcohol and concentrating the extract *in vacuo*. The resulting syrup was brought to a final concentration such that 1 cc. contained the equivalent of 2 grams of the polishings. This tikitiki, fed at the level of 0.5 cc. per animal daily and supplemented with 40 micrograms pure riboflavin per

[3] Two preparations of riboflavin were used: that marketed by the Borden Company, Bainbridge, N. Y., as crystalline lactoflavin, PX grade (highest purity) and a riboflavin obtained from the S. M. A. Corporation, Cleveland, stated to be not less than 96–98 per cent pure.

[4] We are indebted to the Eli Lilly Company for furnishing the rice polishings.

animal daily, was given to 21-day-old rats as the sole source of water soluble vitamins. The animals were enabled to make a good rate of growth to maturity, and were maintained in excellent condition for periods of over a year. The very slow growth of the rats receiving the tikitiki without riboflavin, as well as the symptoms of acute deficiency (loss of hair, growth failure, and death) which appeared in many of the animals, indicated that the preparation contained only a trace of riboflavin.

The tikitiki was assayed for vitamin $B_1$ by the pigeon method used in this laboratory (2) and found to contain 3.3 pigeon units per cc. (approximately 8 international units). It was fed to the dogs in the amount of 4 international units per kilogram of body weight daily, a level furnishing

TABLE 1

*Artificial ration lacking riboflavin*

Basal mixture

| CONSTITUENTS | PERCENTAGE |
|---|---|
| Purified casein* | 30.0 |
| Sucrose | 36.0 |
| Crisco | 26.9 |
| Linoleic acid | 0.1 |
| Bone ash | 4.0 |
| Salt mixture† (Osborne-Mendel) | 3.0 |

This basal ration was supplemented as follows: Tikitiki was given as a source of B vitamins other than riboflavin. It was prepared from rice polish and fed at a level equivalent to 1 gram of rice polish per kilogram of body weight daily. Vitamins A and D were provided in the form of cod liver oil concentrate (furnished by the White Laboratories, Health Products Corporation, Newark, N. J.) to the extent of 2 tablets per dog daily.

* Labco brand casein, obtained from the Borden Company, Bainbridge, N. Y.

† The original salt mixture (9) was modified by the addition of 0.75 gram of $CuCl_2 \cdot 2H_2O$ and 0.68 gram of $MnSO_4 \cdot 2H_2O$ to the proportion of reagents listed by Osborne and Mendel.

three times the amount of vitamin $B_1$ estimated to be required by dogs of this size (4).

*Grouping of animals.* The 11 dogs used in this study are best described as belonging to three groups.

Group 1: Six dogs were given the basal diet alone until the appearance of the collapse syndrome. They were then treated with a single dose of pure riboflavin, and, if recovery occurred, were continued on the basal diet until the second collapse. Each dog was offered daily an amount of the basal mixture furnishing approximately 90 calories per kilogram, an amount sufficient to permit a small gain in weight if completely consumed.

Group 2: This group contained a single animal, dog 4. This animal was one of a group that received small daily doses of riboflavin (4 to 8 micrograms per kilogram of body weight) which were insufficient to maintain complete health. It was not expected that acute riboflavin deficiency would appear in this group; however, this one dog developed the characteristic collapse syndrome when the daily riboflavin dosage was reduced from the level of 8 to that of 4 micrograms per kilogram.

Group 3: This group consisted of four dogs that served as inanition controls. They were given the basal diet plus 25 micrograms of riboflavin per kilogram of body weight daily. Three of these dogs were each paired with an animal in group 1 of approximately the same weight. One animal was an inanition control for dog 4 in group 2. The control animal received each day the amount of the basal mixture eaten by the experimental partner the preceding day. Thus, the only difference in the treatment of the control dogs and of their experimental mates was in the amount of riboflavin given.

*Clinical observations.* The dogs were weighed weekly. At frequent intervals they were examined for any evidences of abnormality, particular attention being paid to the mouth, eyes, coat, and skin. Careful records were kept of such symptoms as vomiting and diarrhea. Complete necropsies of the animals were performed immediately after death. Each control animal was chloroformed and autopsied when its experimental mate died.

Results. *General behavior of the animals.* After a variable period of time on the diet (1 to 9 weeks) there began a steady decline in the body weights of the dogs receiving the basal diet only. In all cases the loss in body weight was accompanied by a decrease in food consumption.

The partial loss of appetite in these animals bears many points of difference from the marked anorexia that appears in animals deprived of vitamin $B_1$ (3). The significant features of the decreased appetite appearing in riboflavin deficiency are summarized in the following data taken from the records of five of the dogs in group 1 (dog 2 is omitted because of its somewhat anomalous behavior): The decrease in appetite appeared in from 2 to 11 weeks after the animals were placed on the diet, and from 8 to 13 weeks before they collapsed. The weekly food consumptions did not as a rule become progressively less, but fluctuated in a rather random manner, and never dropped to zero. About half of the dogs continued to eat until the beginning of the collapse syndrome, whereas the remainder stopped eating one to two days before.

A typical example of the course of the appetite in this experiment is seen in the food consumption per week of dog 3 from the onset of decreased appetite until the onset of fatal collapse. The amounts ingested volun-

tarily per week were 71, 60, 78, 74, 77, 79, 50, and 57 per cent of the food offered.

The appetites of the dogs on the riboflavin-deficient diet may be contrasted with those of a group of five dogs we have observed on a diet lacking vitamin $B_1$. A decrease in appetite appeared in these dogs from 4 to 27 days before the development of acute polyneuritis. The appetite became progressively poorer until anorexia was nearly or quite complete for an average of 7 days (range 3 to 9) before prostration. Three of the dogs ate nothing during this period, one dog ate a total of 50 grams of food in 7 days. Another dog, which in health consumed 210 grams of food daily, ate a total of 90 grams of food in the 9 days preceding polyneuritis. The administration of synthetic crystalline vitamin $B_1$[5] to these dogs resulted in prompt and complete restoration of appetite.

Accompanying the loss of body weight of the dogs in group 1 there was some decrease in activity. Aside from this there were no other notable external symptoms of abnormality. Diarrhea has been a prominent feature of the effects that previous investigators have reported in canine studies with diets lacking heat stable factors of the vitamin B complex; in particular, diarrhea is a prominent symptom of canine blacktongue produced with the Goldberger diet (6) and of the disease produced by the vitamin G deficient ration of Zimmerman and Burack (14). In the present study diarrhea was only very infrequently observed, except that it was usually present during the syndrome of collapse and sometimes for one or two days preceding.

Likiwise, vomiting was sometimes seen, but very infrequently.

In contrast to the group of experimental dogs, the four controls, receiving the basal diet plus 25 micrograms of riboflavin per kilogram, remained in perfect health throughout the experiment. They did, however, lose considerable weight because the food consumption of each control animal was limited to that of the experimental partner receiving the basal diet only. These observations, therefore, indicate that a simple inanition of varying degree cannot account for the abnormal behavior of the riboflavin-deficient animals.

*The characteristic collapse.* On the basal diet unsupplemented with riboflavin, the initial collapse in the six experimental dogs occurred in 102, 109, 112, 138, 140, and 248 days, respectively. The extended time required for this latter animal is partly explained by the fact that through a mistake it received a diet containing 8 per cent yeast for five days, beginning on the 157th day. One dog, given daily subminimal doses of riboflavin (4 to 8 micrograms per kilogram of body weight), collapsed after 332 days.

[5] Thiamine chloride (crystalline) obtained from the Winthrop Chemical Company New York, N. Y.

Occasionally, but not always, the animal's appetite failed completely for a short time preceding the sudden failure; in six of the ten instances observed, the dog ate no food for one to two days preceding. Aside from this there was very little warning of the approaching collapse. The progress of the syndrome is usually about as follows: the dog is found unusually quiet and apathetic, often reluctant to walk. The gait soon becomes staggering, and a moderate stiffness of the hind legs develops. The weakness increases so that the animal when put on its feet refuses to walk. A little later it is unable to stand and when put on its feet either slumps down or falls over. It then passes into a semicomatose condition in which it will make no effort whatever to stand, although it is able to raise its head. Following this, if no treatment is given, the animal passes into deep coma and dies within one or two to several hours.

Some animals undergo intermittent convulsions after they are unable to stand. Vomiting almost always occurs; sometimes there are periods of continued retching. There is usually diarrhea.

The rate of progress of the attack is extremely variable. In five cases in which the animals were watched with great care for the first symptoms, the condition was diagnosed 3, 5, 13, 31, and 35 hours before the animal was unable to stand.

*Treatment with riboflavin.* In seven instances the dogs were treated with crystalline riboflavin. In six cases this was given in doses approximating 0.75 mgm. per kilogram (estimated to be about 30 times the daily requirement), and in one case half this amount. This was given in two approximately equal portions, the first intraperitoneally, the second subcutaneously. The injection was usually made at the time that the dog was unable to stand, but was not yet comatose. In five of the seven experiments the injected riboflavin restored the animal to an outwardly normal condition within three or four to 24 hours. In the other two instances death occurred in spite of treatment. Within two or three days after treatment the dogs regained most of the vigor and activity they had shown when first placed on the diet. The appetite always improved and remained good for a longer or shorter time. Accompanying the improved appetite there was maintenance or increase of body weight in contrast to the steadily falling body weight before the administration of riboflavin.

On the four dogs continued on the basal diet without further supplement, three collapsed for the second time after 41, 50, and 69 days respectively. The fourth animal, dog 5, which was very thin at the time of the first collapse, recovered the ability to stand after treatment with riboflavin, but remained weak and died 41 hours later. It did not die in the coma characteristic of acute lack of riboflavin. Its body temperature was a little above normal half an hour before death, which may be contrasted

with the very low premortal temperatures of dogs dying after a characteristic collapse. Autopsy failed to reveal any obvious cause of death.

The significant data with regard to the collapse syndrome in the various dogs, and the response to treatment, are summarized in table 2.

*Changes in body temperature, respiration, and heart rate.* In the latter part of the study it was observed that there was a marked fall in body temperature during the periods of collapse. We have found that normal dogs seldom have a rectal temperature falling outside the range 38–39°. The drop in temperature did not begin before physical symptoms, as inactivity or staggering, revealed the initial stage of an acute attack. The

TABLE 2

*A summary of the effect of the administration of riboflavin to dogs in the characteristic collapse appearing on a diet lacking riboflavin*

| DOG NUMBER | BODY WEIGHT | | | | TIME OF COLLAPSE (DAY OF EXPERIMENT) | RIBOFLAVIN GIVEN | CONDITION WHEN TREATED | RESULT |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | At time of treatment | | | | | |
| | | | Kilos | Per cent of initial weight | | | | |
| | *kilos* | *kilos* | | | | *mgm.* | | |
| 1 | 8.6 | 6.0 | 6.5 | 76 | 102 | 5.25 | Unable to stand | Recovered |
| | | | | | 152 | | | Not treated |
| 2 | 7.3 | 9.0 | | | 248 | | | Not treated |
| 3 | 7.3 | 5.6 | | 77 | 112 | 5.25 | In deep coma | Died |
| 4* | 6.0 | 5.9 | 4.9 | 82 | 332 | 2.25 | Unable to stand | Recovered |
| 5 | 6.0 | 3.6 | 4.0 | 67 | 109 | 3.75 | Unable to stand | Recovered |
| 6 | 7.9 | 5.3 | 5.6 | 71 | 140 | 4.50 | Unable to stand | Recovered |
| | | | | | 181 | | | Not treated |
| 7 | 7.4 | 4.8 | 5.3 | 72 | 138 | 4.50 | Falls frequently | Recovered |
| | | | | | 207 | 4.00 | Staggers slightly | Died |

* Received 4 to 8 micrograms of riboflavin per kilogram daily throughout the experiment.

temperature then fell steadily as the syndrome progressed, but began to rise again as soon as riboflavin was given, providing that treatment was not started too late. The data for these temperature changes are shown in tables 3 and 4.

In two of the dogs (animals 6 and 7) the pulse and respiration rates were followed during the periods of collapse, and these are also summarized in tables 3 and 4. The respiratory rate was consistently lowered from the usual level of 15–20 per minute to rates of one-half or one-third the normal. This decreased respiratory rate occurred abruptly at the beginning of the collapse syndrome, as though it were a primary feature of the condition. Thus, for dog 7 on May 29 (see table 4) the temperature and rate

of respiration were found to be 38.1° and 12 per minute, respectively, while eight hours later the temperature was 37.6° (0.4° below the normal range) and the rate of respiration was 5 per minute. This drop in respira-

TABLE 3

*Observations on dog 6 during periods of collapse*

| DATE | TIME | BODY TEMPERATURE | RESPIRATION | HEART RATE | BLOOD* GLUCOSE | PHYSICAL CONDITION |
|---|---|---|---|---|---|---|
| | | °C. | *per min.* | *per min.* | *mgm./ 100 cc.* | |
| | | | | | | First collapse |
| | 9:30 a.m. | | | | | Inactive, weak |
| | 12:00 m. | | | | | Stands with difficulty |
| | 1:00 p.m. | | | | 35 | |
| | 2:25 p.m. | 34.8 | | | | Unable to stand |
| | 3:30 p.m. | | | | | 4.5 mgm. riboflavin injected |
| Feb. 4 | 3:40 p.m. | | | | 39 | Semicomatose |
| | 4:10 p.m. | 34.4 | | | | Convulsing |
| | 5:25 p.m. | 35.4 | | | | Holds its head up |
| | 6:10 p.m. | 35.7 | | | | Walks in nearly normal manner |
| | 9:15 p.m. | 38.0 | | | | Further improved |
| Feb. 5 | | 38.8 | | | | Normal |
| Feb. 7 | | | | | 105 | Normal |
| Mar. 15 | | 38.3 | | | | Essentially normal |
| Mar 16 | | 38.1 | | | | Essentially normal |
| | | | | | | Second collapse |
| | 5:00 p.m. | 36.9 | | | | Inactive |
| Mar. 17 | 6:00 p.m. | | | | 102 | Staggers slightly |
| | 9:15 p.m. | 36.5 | | | | Staggers slightly |
| | 11:00 p.m. | 36.5 | | | 133 | Staggers slightly |
| | 2:45 a.m. | 36.5 | | | | Staggers slightly |
| | 6:30 a.m. | 36.2 | | | | A little weaker |
| | 9:15 a.m. | 36.0 | 9 | 136 | | Walks with marked stagger |
| | 12:00 m. | 35.9 | 10 | 124 | | Staggers and falls |
| Mar. 18 | 2:00 p.m. | 35.9 | 8 | 128 | | Stands but will not walk |
| | 4:45 p.m. | 36.0 | | 76 | | Stands a short time, then falls |
| | 6:30 p.m. | | 10 | | | Stands a short time, then falls |
| | 11:15 p.m. | 33.5 | | | | Moribund |
| Mar. 19 | 1:00 a.m. | | | | 119 | 30 minutes post mortem |

* Blood glucose was determined by the Shaffer-Hartmann method (10), using zinc precipitation of proteins.

tion was not simply an expression of a moribund state, as the dog at this time showed only a slight change from its usual behavior, and its general condition appeared to be good.

TABLE 4

*Observations on dog 7 during periods of collapse*

| DATE | TIME | BODY TEMPERATURE | RESPIRATION | HEART RATE | BLOOD GLUCOSE | PHYSICAL CONDITION |
|---|---|---|---|---|---|---|
| | | °C. | *per min.* | *per min.* | *mgm./100 cc.* | |
| Mar. 21 | | 38.7 | | | | Essentially normal |
| Mar. 22 | | 38.9 | | | | Essentially normal |
| | | | | | | First collapse |
| | 11:25 a.m. | 37.4 | | | | Staggers badly |
| | 11:50 a.m. | | | 72 | 35 | |
| | 12:40 p.m. | 36.8 | | 76 | | Staggers and falls |
| | 1:40 p.m. | 36.7 | | | | Staggers |
| | 2:00 p.m. | | | | 39 | Staggers |
| Mar. 23 | 3:10 p.m. | 37.2 | | | | 4.5 mgm. riboflavin injected |
| | 3:30 p.m. | | | | 63 | Sways in walking |
| | 4:45 p.m. | 37.7 | | | | |
| | 5:55 p.m. | 38.1 | | | | Stands but will not walk |
| | 10:15 p.m. | 38.3 | | | 109 | Walks in normal manner |
| Mar. 24 | | 38.0 | | 76 | 112 | Essentially normal |
| May 7 | | 38.5 | 16 | 76 | | |
| May 13 | | 38.5 | | 104 | 88 | Normal |
| | | | | | | Fall in temperature with spontaneous recovery |
| | 12:30 p.m. | 37.6 | 10 | 80 | | Quiet, sways in walking |
| | 1:15 p.m. | | | | 63 | |
| May 15 | 3:45 p.m. | 37.4 | | | 84 | Quiet, sways in walking |
| | 8:00 p.m. | 37.9 | 8 | 112 | 76 | A little more active |
| May 16 | 6:00 a.m. | 38.1 | 7 | 104 | 71 | Essentially normal |
| May 25 | | 38.0 | 16 | 96 | 87 | Essentially normal |
| | | | | | | Second collapse |
| | 11:30 a.m. | 38.1 | 12 | 92 | | A little quieter |
| May 29 | 7:15 p.m. | 37.6 | 5 | 96 | | Sways slightly |
| | 7:55 p.m. | | | | 53 | |
| | 1:35 a.m. | 37.0 | 5 | 148 | 46 | Condition the same |
| | 10:00 a.m. | 37.0 | 5 | 92 | 49 | Condition the same |
| | 12:30 p.m. | 36.5 | 4 | 146 | 61 | Quieter, staggers |
| May 30 | 2:35 p.m. | 36.0 | 6 | 168 | 79 | Condition the same |
| | 6:00 p.m. | 36.0 | | 164 | | Condition the same |
| | 11:50 p.m. | 36.0 | 6 | 160 | 87 | Condition the same |
| | 5:00 a.m. | 33.9 | 6 | 116 | 72 | Staggers markedly |
| | 5:50 a.m. | 33.8 | 6 | 104 | 51 | 4 mgm. riboflavin injected |
| May 31 | 6:05 a.m. | 33.4 | | 96 | | Staggers markedly |
| | 7:00 a.m. | 32.9 | 7 | 88 | 67 | Unable to stand |
| | 8:35 a.m. | | | | 27 | 5 minutes post mortem |

The heart rate was either normal or high in the acute attacks in dogs 6 and 7. Electrocardiograph records taken of these two animals during collapse, and at several other times during the experimental period, for

the most part, did not reveal anything unusual. However, there was in both dogs an inversion of the T-wave during the acute attacks, whereas at all other times the T-wave was upright. Thus, in dog 6, there was an inverted T-wave in two records taken during the first collapse, whereas, on the following day, after recovery following riboflavin administration, the T-wave was upright. Sebrell and Onstott (12) have also observed this inversion of the T-wave in acute riboflavin deficiency.

*Additional observations.* During the entire experimental period the dogs remained free from any symptoms of blacktongue, such as excessive salivation or reddening of the oral mucosa. There was no loss of hair, such as is seen in rats on diets lacking riboflavin.

Cataract, which has been found in young rats on a diet lacking riboflavin, did not appear in these dogs, at least not in a form visible grossly.

Periodic determinations of the blood hemoglobin made on three dogs of group 1 revealed consistently normal values for dog 5, while dogs 6 and 7 developed a moderate degree of anemia.

Autopsies performed on the dogs dying from acute riboflavin deficiency for the most part disclosed nothing abnormal. There was a pneumonic process in the lungs of two of the animals, small in extent in one, and involving most of the right lung in the other. The liver of dog 1 was of a slightly yellowish cast, and that of dog 5, the yellow-brown color which is spoken of as a "yellow liver." The livers of the other dogs did not appear unusual. The rest of the viscera in this group of animals presented a normal appearance.

The autopsies of the control dogs in group 3, which were chloroformed, in all cases revealed normal appearing viscera.

Discussion. From the data accumulated in this study it is possible to make some suggestions as to the cause of the characteristic collapse. It is certainly not due simply to general inanition, since, as indicated, in table 2, the body weights of the dogs at the time of the first collapse were from 67 to 82 per cent of the initial body weight, with the exception of one dog which gained weight. In general, animals on deficient diets undergo larger losses of weight than this before failure from inanition occurs. Moreover, in this study, the control dogs lost comparable amounts of weight, yet remained in good health.

The fall in body temperature appears to be secondary to the decrease in the rate of respiration, since, as shown in tables 3 and 4, there was a marked fall in the respiratory rate at the beginning of the collapse syndromes before there was an appreciable fall in body temperature. The known function of riboflavin as a component of Warburg's yellow enzyme, which is a member of an oxidation-reduction system (13), suggests an explanation for these effects. It seems possible that the decrease in re-

spiratory rate and the fall in body temperature result from a decrease in the metabolic rate occasioned by a deficiency of the yellow enzyme.

The heart rate, in the three acute attacks in which it was followed, was normal or rapid. Thus, we do not confirm the conclusions of Sebrell and Onstott (12) that this condition in dogs is characterized by bradycardia. Moreover, an analysis of their data reveals that their animals did not consistently show a bradycardia during the acute attacks of riboflavin deficiency.

In several respects the symptoms of this syndrome of collapse are similar to those seen in the development of hypoglycemic shock; in particular, the gradually developing weakness and apathy, and the convulsions sometimes seen, are suggestive of a hypoglycemic reaction. Blood glucose analyses made in four acute attacks reveal, however, that while blood sugar values as low as 35 to 40 mgm. per 100 cc. are occasionally seen, the values are not consistently low and do not show any constant trend during the attacks.

The main features of the syndrome are apparently not due to a lowering of blood pressure from a concentration of the blood, since a series of blood samples taken from the femoral arteries of two dogs during the progress of the terminal syndromes of collapse showed no significant variations in hemoglobin.

The results of this study give some information as to the probable riboflavin requirement of the dog. Thus it was found that the daily dose of 25 micrograms of riboflavin per kilogram of body weight maintained the three control animals for the dogs of group 1 in apparently perfect health for 130 to 196 days. Because of the long storage period for riboflavin this does not indicate with certainty that this dose completely meets the animals' requirements. However, in another study which will be reported in detail later, three dogs which received daily 25 micrograms of riboflavin per kilogram, in addition to the basal diet used here, remained in excellent health for over 500 days.

## SUMMARY

1. Dogs maintained on a diet containing not more than traces of riboflavin have been found to collapse in a characteristic manner in from 102 to 140 days.

2. The injection of crystalline riboflavin in the amount of 0.75 mgm. per kilogram of body weight to the animals in this collapsed state results in prompt recovery if the injection is not unduly delayed.

3. The collapse is accompanied by a marked fall in body temperature. A limited number of observations indicates that the condition is further characterized by a fall in respiratory rate and by a variable heart rate.

4. Supplementation of the basal riboflavin deficient diet with 25 micro-

grams of riboflavin per kilogram of body weight has been found to maintain dogs in health for an extended period.

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# THE REPRODUCTIVE PERFORMANCE OF ALBINO RATS WITH PREVIOUS VITAMIN A DEPLETION HISTORIES[1, 2]

LUDVIG GUSTAV BROWMAN

*Hull Zoölogical Laboratory, University of Chicago, and Department of Zoölogy, Montana State University*

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It is well known that animals kept on vitamin A deficient diets have difficulty in conceiving, in bearing living young, and in lactating (Evans, 1928; Mason, 1935). If such animals are subsequently placed on rations adequate in vitamin A these difficulties tend to disappear. However it is not known whether recovery is complete or partial, that is, whether any residual injurious sequelae or "latent deficiencies" remain from the vitamin depletion histories. Several workers using pigs as experimental animals with vitamin A deficient diets have reported abnormalities in the eyes, limbs, urinogenital tract, and other organs of the offspring of such depleted animals (Hughes, Aubel and Lienhardt, 1928; Hale, 1933, 1935; Hughes, 1934a, 1934b).

This report presents the results of experiments on rats carried out to secure information on the following questions: 1, what effect does previous depletion of vitamin A have on the reproductive performance of female rats on diets adequate in all respects for reproduction; 2, what effect does previous depletion of vitamin A have on the reproductive performance of female rats maintained on various levels of Beta carotene supplement, and 3, what effect does the use of cotton seed meal in rations low or deficient in vitamin A have on the reproductive performance in rats.

Female rats, 26 to 28 days of age and 40 to 50 grams in weight, were twice depleted of vitamin A.[3] These twice depleted animals were divided at random into groups of six animals each. Each group was given ration B no. 4, plus varying amounts of Beta carotene supplement, as follows: Wesson oil only; 1.5 micrograms of Beta carotene in Wesson oil, 3.0, 4.5, 6.0, and 9.0 micrograms of Beta carotene in Wesson oil daily. Four con-

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[2] The author wishes to express his sincere appreciation for the facilities offered by the Hull Zoölogical Laboratory, and especially Dr. C. R. Moore, which made this work possible.

[3] Ration B no. 4: heated casein 18, tankage 2, heated cornstarch 49, irradiated dried yeast 12, Crisco 8, Wesson oil 7, Wesson '32 salt mixture 4.

trol groups were used: 1, six virgin colony females 3–4 months of age were continued upon the stock ration;[4] 2, six virgin colony female rats, 28 days of age, were placed upon ration B no. 4 plus a daily supplement of 10.4 micrograms of Beta carotene in Wesson oil; 3, six twice depleted females were brought to approximately normal weight with ration B no. 4 plus Beta carotene and were then placed upon the stock ration, and 4, six twice depleted females were placed directly upon the stock ration after the second depletion.

In the cotton seed meal series female colony rats were started on the experimental rations at 28 days of age. These animals were placed in groups of six in large cages with half-inch mesh false bottoms. Weekly weight records beginning at 28 days of age indicated comparatively uniform growth curves on all rations. The average age at vaginal introitus was 42.8 days, and the average length of the first five vaginal cycles was 5.1 days.

The female rats on the experimental and stock diets were placed nightly on attaining 100 days of age with normal males until spermatozoa were found in the daily morning vaginal smear, or until positive pregnancies occurred. Positive pregnancies were diagnosed by means of the presence of erythrocytes in the vaginal smear, by manual palpation, and by rapid weight increase. All young born were routinely examined to ascertain the presence of eye, limb, or other macroscopic abnormalities.

The reproductive performance of six normal control females was arbitrarily selected as representing optimum reproductive performance for this experiment. The reproductive performance of all groups was compared on the same empirical basis with this group as a standard. The experiments were all carried out during the late winter and early spring months so that seasonal factors were not considered to be involved.

Animals never depleted of vitamin A were markedly superior in their reproductive performance as compared to animals previously depleted of vitamin A. That is, females that had been subjected to vitamin A depletion histories were able to carry on only partially successful pregnancies and lactation, although they did show a constant growth and weight increase as soon as a source of vitamin A was provided.

This was true whether the source of vitamin A was in the stock ration or as a Beta carotene supplement to the ration B no. 4. In spite of subminimal doses of Beta carotene on the lower levels no gross abnormalities occurred in any of the young.

French and Bloomfield (1937) reported that twice depleted rats showed a more rapid loss of weight during the second depletion than during the

[4] Stock ration B no. 5: ground whole wheat 36, ground whole yellow corn 10, commercial casein 8, powdered whole milk 15, tankage 2, alfalfa meal 6, irradiated dried yeast 8, Wesson oil 6, Crisco 4, cod liver oil 2, Wesson '32 salt mixture 3, fresh lettuce leaves twice weekly.

first, and suggested that some "real alteration in the status of the animals" had occurred. Similarly it is probable that an altered physiological condition exists in twice depleted animals returned to an adequate source of vitamin A which is responsible for their poor reproductive performance as compared to that of animals never depleted and on identical rations.

TABLE 1

*Summary of the reproductive performance of rats twice depleted of vitamin A and then supplied with various levels of vitamin A*

| | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 | GROUP 6 | GROUP 7 | GROUP 8 | GROUP 9 | GROUP 10 | GROUP 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. times depleted | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Micrograms daily, Beta carotene | 0 | 10.4 | 0 | 0 | 9.0 | 6.0 | 4.5 | 3.0 | 1.5 | oil | 0 |
| Ration ad lib. | B5 | B4 | B5 | B5 | B4 | B4 | B4 | B4 | B4 | B4 | B4 |
| Number rats | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Number pregnancies | 6 | 6 | 6 | 5 | 5 | 4 | 4 | 4 | 5 | 0 | 0 |
| Number litters born | 6 | 6 | 6 | 5 | 5 | 4 | 3 | 2 | 2 | 0 | 0 |
| Total number born | 55 | 48 | 48 | 30 | 28 | 20 | 18 | 8 | 5 | 0 | 0 |
| Number born alive | 54 | 46 | 35 | 29 | 26 | 16 | 15 | 7 | 3 | 0 | 0 |
| Average size litters at birth | 9.1 | 8 | 8 | 6 | 5.6 | 5 | 6 | 4 | 2.5 | 0 | 0 |
| Number young weaned | 42* | 33 | 14 | 24 | 18 | 8 | 9 | 5 | 0 | 0 | 0 |
| Average size litters at weaning | 7 | 5.5 | 2.3 | 4.8 | 3.6 | 2 | 3 | 2.5 | 0 | 0 | 0 |
| Number females lactating | 6 | 5 | 4 | 5 | 4 | 3 | 3 | 1 | 1 | 0 | 0 |
| Number females weaning young | 6 | 4 | 4 | 5 | 3 | 3 | 3 | 1 | 0 | 0 | 0 |
| Number females dying | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 6 | 6 |
| Number eye abnormalities | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Per cent rats pregnant | 100 | 100 | 100 | 83 | 83 | 66 | 66 | 66 | 83 | 0 | 0 |
| Per cent pregnant rats littering | 100 | 100 | 100 | 100 | 100 | 100 | 75 | 50 | 40 | 0 | 0 |
| Per cent young born alive | 98 | 96 | 73 | 98 | 96 | 80 | 83 | 88 | 60 | 0 | 0 |
| Per cent available young weaned | 100 | 72 | 40 | 82 | 69 | 50 | 20 | 71 | 0 | 0 | 0 |
| Reproductive success | 100 | 92 | 58 | 68 | 59 | 38 | 37 | 25 | 20 | 0 | 0 |

* Litters reduced to 7 at birth.

No eye, limb, or other macroscopic abnormalities were observed in the 260 young rats born to females on low levels of vitamin A supplement. The two cases of microphthalmia that did occur were in young born to a non-depleted rat on the stock ration and to a twice depleted rat on a high level of Beta carotene supplement. Since occasional microphthalmic animals normally occur in the colony (about 0.7 per cent) it is unlikely that the two cases of microphthalmia which occurred were due to vitamin A deficiency.

Rations containing amounts of cotton seed meal as high as 80 per cent with only 0.2 per cent supplement of cod liver oil did not induce abnormalities in the young. In other words, the presence of high percentages of cotton seed meal in rations otherwise adequate for reproduction had no deleterious effects upon the reproductive performance of female rats, although their body weight was somewhat subnormal. Rations to which no supplement of vitamin A was added (other than that present in the natural foods) resulted in subnormal reproductive performance but did not induce abnormalities in the offspring.

The only gross abnormalities noted in the 522 young born to animals in the cotton seed meal series were four cases of microphthalmia.

## SUMMARY

Rats with previous vitamin A depletion histories have reproductive performances which are definitely subnormal to the reproductive performance of animals never depleted, when the diet is adequate in all respects for reproduction.

Rats maintained on the various levels of Beta carotene supplement following depletion of vitamin A have reproductive performances definitely below those of animals never depleted.

Rats maintained on diets containing high percentages of cotton seed meal and low in vitamin A have reproductive performances that approach normal reproductive performances.

Only six young of the 782 offspring born to females in this report showed gross abnormalities. All of these abnormalities were microphthalmia. But since microphthalmia occurs normally in approximately 0.7 per cent of the young born in this colony, the presence of high per cent of cotton seed meal, vitamin A depletion histories, and maintenance of previously depleted rats on low levels of Beta carotene can not be said to have induced the appearance of microphthalmia.

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# FORCES AND ENERGY CHANGES IN THE LEG DURING WALKING

HERBERT ELFTMAN

*From the Department of Zoology, Columbia University, New York City*

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The study of locomotion yields information not only concerning the mechanics of progression but also concerning the part that muscles play in this intricate activity. Early attempts at a quantitative analysis of human locomotion were made by the Webers, Marey and Otto Fischer, followed in recent years by Bernstein (1927, 1935) and by Fenn (1929, 1930). The present paper is concerned with a detailed analysis of the dynamics of the human leg in walking, providing data concerning muscle function.

In his fundamental investigations of the kinematics of walking, Fischer (1901) was hindered by the fact that he could not determine the point of application of the force exerted by the ground on the foot. The path of this point during the time the foot is on the ground was determined by Elftman and Manter (1934) from cinematic records of pressure distribution in the foot, obtained by a method described by Elftman (1934). For the purposes of the present research, however, a new apparatus has been devised (Elftman, 1938), which gives not only the point of application of this force, but also the magnitude of the force in three components. In addition to solving Fischer's difficulty, this obviates the necessity of dealing with the entire body when only one portion, such as the leg, is of immediate interest.

The point of application of this force, as it passes forward during the course of the step, is shown in figure 1. The force itself is plotted in two components, one in the plane of progression, the other lateral, in the horizontal plane. Only the component in the plane of progression will be considered in the present discussion. It is apparent from the diagram that it has two maxima and that it is at first directed upward and backward against the foot, later upward and forward. This reaction of the platform is plotted in figure 6 in two components, one vertical and the other horizontal, in the plane of progression.

In addition to this knowledge concerning the external force exerted by the platform, it is necessary to know the disposition of the leg in space. This is shown in figure 2 for the left leg during the double step under con-

sideration. The original information was obtained from cinematic records taken at the rate of 92 exposures per second as the subject walked behind a rectangular grid. The timing was obtained by including a vibrating reed

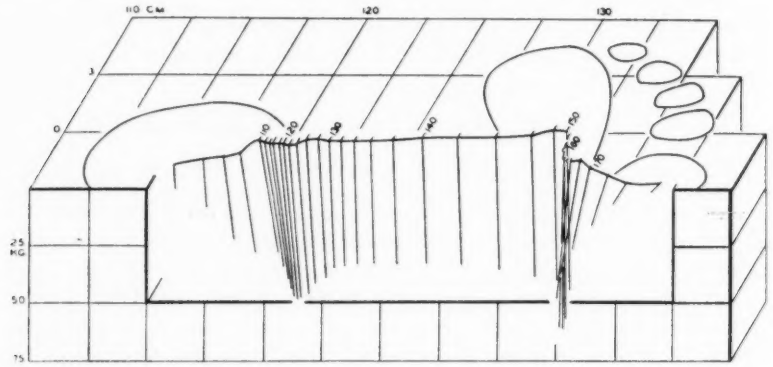


Fig. 1. Reaction of the platform on the foot and its point of application. The reaction is shown in two components, one in the plane of progression, the other lateral, in the horizontal plane.

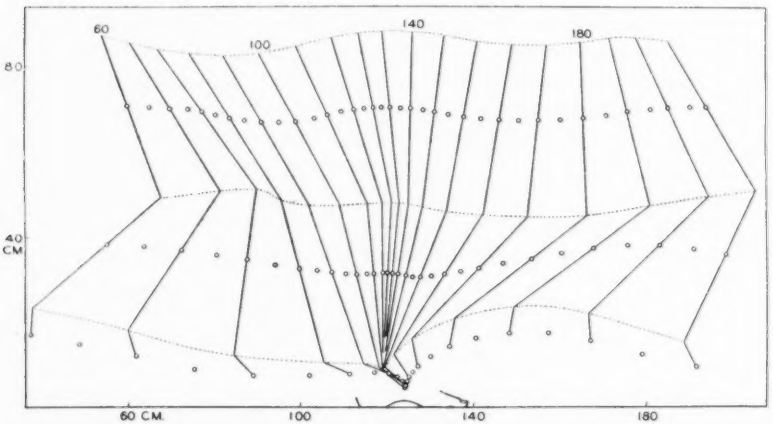


Fig. 2. Position of the left leg during a double step. The centers of gravity of the thigh, shank and foot are indicated by circles. Time interval between successive positions of the leg, 0.08 sec. The phases are numbered in hundredths of a second, the time of application of the left foot to the platform being taken as 100.

of known period in the photographic field. These records were projected on a large sheet of paper and the positions of the axes of the hip-, knee- and upper ankle-joints determined. After plotting these positions against

time, values were read off at intervals of 0.02 sec. and these were used in all further calculations. The phases are numbered according to the time at which they occurred in hundredths of a second, taking the time of establishment of contact by the foot with the platform as 100. In figure 2 the positions of the axes of the limb are shown at intervals of 0.08 sec. The circles indicate the positions of the centers of gravity of the thigh, shank and foot. They have been determined by applying the proportions

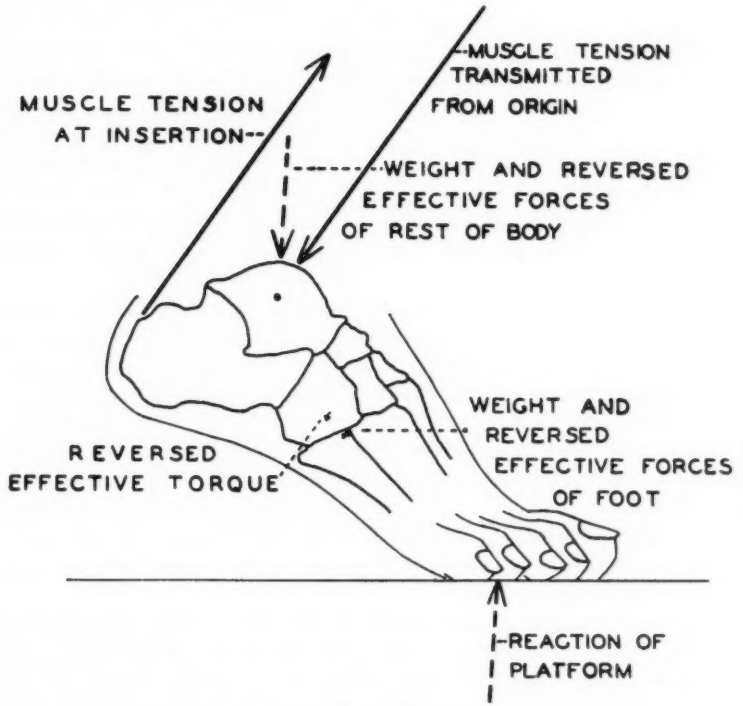
MUSCLE TENSION AT INSERTION
MUSCLE TENSION TRANSMITTED FROM ORIGIN
WEIGHT AND REVERSED EFFECTIVE FORCES OF REST OF BODY
WEIGHT AND REVERSED EFFECTIVE FORCES OF FOOT
REVERSED EFFECTIVE TORQUE
REACTION OF PLATFORM

Fig. 3. Free body diagram of foot

given by Fischer (1906) for their positions with respect to the adjacent joints.

*Determination of forces and torques.* The external force and the position of the leg, as portrayed in figures 1 and 2, constitute the fundamental observed data, from which other information, more essential to our purpose, may be derived. The next step is to determine the forces acting on the foot. These are shown in figure 3, which is a free-body diagram of the foot. According to D'Alembert's principle, the reversed effective forces

must be in equilibrium with all other forces acting on the body. Consequently if all the forces are known with one exception, that exception can be solved. Upon inspection of the forces, we find that the reaction of the platform is known (figs. 1 and 6). The weight of the foot is known to be 1.14 kgm. by applying Fischer's proportions to the total body weight of 63.4 kgm. The reversed effective force is equal to $-ma$, when $m$ is the mass of the foot in gravitational units and $a$ the acceleration of the center of gravity of the foot. The acceleration is obtained by plotting displacement against time and differentiating graphically, using the prism method, obtaining the velocity. The process is then repeated with the velocity to get the acceleration. The reversed effective force can therefore be calculated; it is plotted in figure 5.

The only force left which is not a member of a couple is the one which acts through the ankle-joint, due to the weight and reversed effective forces of the rest of the body. Since this force must be in equilibrium with the forces already known, it is readily calculated. This ankle-joint force is shown in figure 6, plotted, however, as it acts on the shank, which is opposite to its effect on the foot.

With all the forces determined, it is possible to approach the torques. Since the positions of the forces are known, their lever arms about any desired point can be measured and so their moments, or torques, about that point determined. The reversed effective torque is calculated in a manner analogous to that used for reversed effective forces. The moment of inertia, calculated from Fischer's data, is 0.046 kgm. slug cm.$^2$ for the foot. The angular acceleration is obtained by double differentiation of the angular displacement. The reversed effective torque in walking is quite small; it is plotted in figure 7. The only torque still unknown is that of the muscles, consequently that can be found by solving the equations for equilibrium.

With the forces and torques acting on the foot completely determined, it is possible to repeat the operations with the shank and the thigh. This is possible because the joint force acting on the member above the joint is equal in magnitude but opposite in direction to the force acting on the member lying below the joint.

The uppermost curve of figure 7 shows the torque exerted by the leg muscles on the trunk. It is possible to calculate this torque because the sum of the torques exerted by any muscle must be zero. This is illustrated for a one-joint muscle in figure 4. The muscle under a tension $F$ will exert a force of $+F$ at its origin and $-F$ at its insertion. When the two members $AB$ and $BC$ upon which the muscle acts are considered separately, it is found that the force $-F$ exerted at the insertion is transmitted to $AB$ through the joint $B$. Since the force on $AB$ at the origin is $+F$ and at the joint $-F$, the two forces acting together constitute a

couple, the torque of which is obtained by multiplying the tension by the perpendicular distance between the two forces. It is obvious from the diagram that the torque on $BC$ is equal in magnitude but opposite in direction to that on $AB$. The sum of the two torques is consequently zero. The same proof may be elaborated for muscles which traverse more than one joint. Consequently the torque exerted by the leg muscles on the trunk must be such that when it is added to the sum of the torques exerted on the foot, shank and thigh the result is zero.

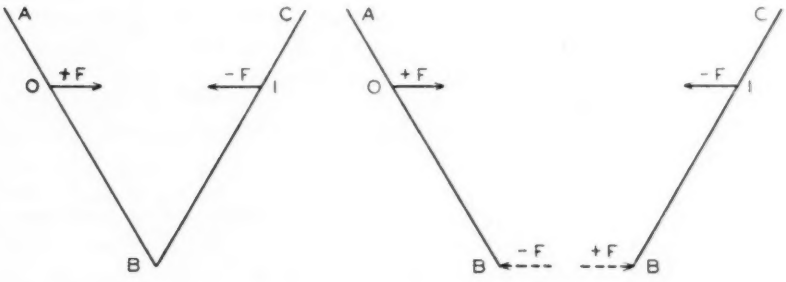


Fig. 4. Diagram of one-joint muscle. The member $AB$ is acted on by the force $+F$ of the muscle at its origin. The force $-F$ exerted by the muscle at its insertion is transmitted to $AB$ through the joint. The two forces constitute a couple.

*Significance of the forces and torques.* The reversed effective forces vary with changes in the momentum of the member, since

$$\text{reversed effective force} = -ma$$

$$\text{and} \quad -ma = -\frac{dmv}{dt}$$

The reversed effective force is consequently the force exerted by the member on other parts of the system due to a decrease in the momentum of the member. When the momentum of the member is increasing, the reversed effective force is negative. The rate of change of linear momentum of the parts of the leg can consequently be read from figure 5. While the left foot is in contact with the ground, the horizontal momentum of all parts of the leg first decreases and then increases. The vertical momentum varies in a less regular fashion. The time during which the foot is stationary on the ground is indicated by the fact that the reversed effective forces of the foot are zero.

The platform reaction, figure 6, represents the only external force acting on the body while the left foot alone is in contact with the substratum, since air resistance may be neglected at the low velocity of walking. The

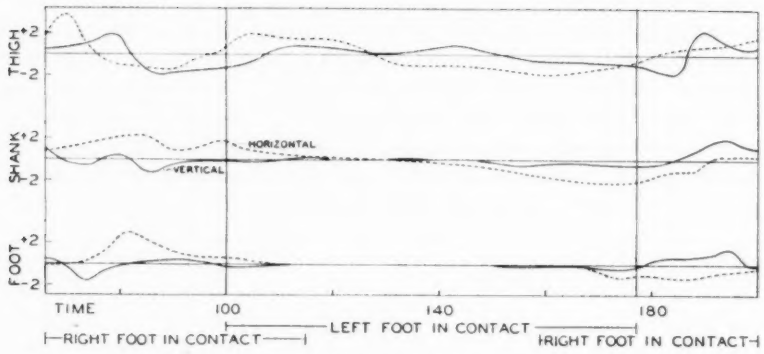


Fig. 5. Reversed effective forces in kgm. Vertical component, continuous line; horizontal component, dotted.

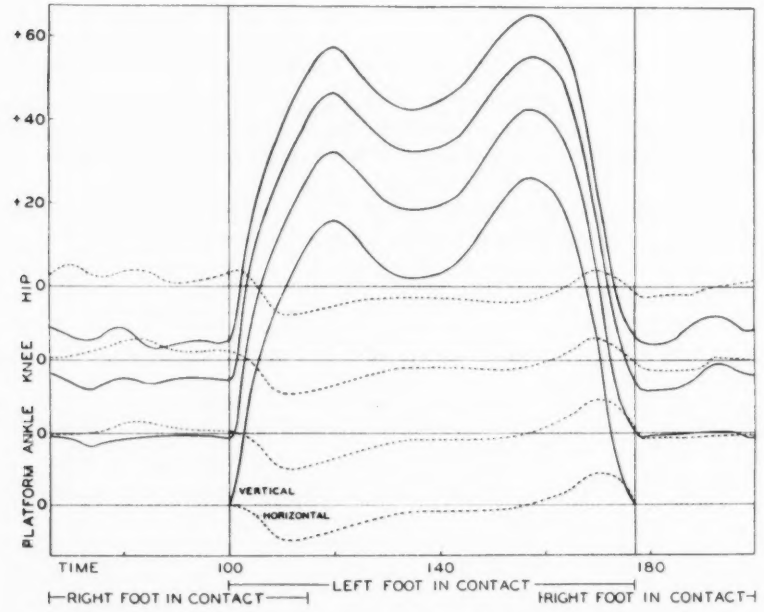


Fig. 6. Platform reaction and the joint forces which are due to the platform reaction, gravity and effective forces. Forces in kgm. The forces plotted are those which act on the member above the joint. The force on the member below the joint is of the same magnitude but opposite in direction. Vertical forces, continuous line; horizontal forces, dotted.

horizontal component of this reaction is at first negative, pushing backwards against the body, and is later positive. This may also be seen in figure 1. The vertical component includes the reaction to gravity as well as the reaction to the effective forces transmitted through the left leg. The reaction to gravity is equal to the weight of the body, but is directed upward, for the interval during which the left foot alone is in contact. By subtracting this value from the total vertical reaction, the portion due to acceleration of the body as a whole may be determined and from this, by integration, changes in the momentum of the body as a whole.

The vertical force exerted by the platform describes a characteristic bimodal curve. Both maxima exceed the value for the reaction to gravity, while the intervening minimum is lower than this value.

The forces acting at the joints, due to the platform reaction, gravity and effective forces, are plotted in figure 6 as they act on the member above the joint. There is, of course, an equal but oppositely directed force which acts on the member below the joint. The force plotted here is not the total force acting through the joint, since it does not include the muscle forces. For purposes of dynamics the effect of the muscle forces can best be obtained from a consideration of the couples by means of their torques. If our purpose were the investigation of pressure in the joints, the procedure would be different.

The joint forces for the interval during which the foot is in contact with the platform vary, in general, in the same way as the platform reaction, since this is the largest component entering into their composition. Each vertical component is decreased, as we ascend from the foot to the thigh, by the weight of the parts of the leg which lie below the joint under consideration as well as by the effective forces of these parts. The joint forces are present while the foot is off the ground, at which time the platform reaction is absent.

The reversed effective torques are shown in figure 7 in dotted lines, when they are of sufficient magnitude to be plotted. A positive reversed effective torque represents the rate at which the angular momentum of the member about its own center of gravity is decreasing. In walking, the reversed effective torques are so small that they could be disregarded during the interval of contact of the foot with the ground. In the present research they have been included in all calculations.

The torques of the reversed effective forces and joint forces are not plotted in figure 7. Since they must be in equilibrium with the muscle torques and reversed effective torques, they must, at each instant, be equal in magnitude to the sum of the muscle and reversed effective torques, but of opposite sign.

The muscle torques, figure 7, represent the resultant torque of all muscles acting on the member. When antagonistic muscles are in action simul-

taneously, the algebraic sum of their torques will be equal to this resultant torque. The torques are plotted in the usual fashion, a positive torque tending to produce counter-clockwise acceleration of the parts of the leg as they are oriented in figure 2. If the leg behaved as a conventional compound pendulum, there would be no muscle torques during its swing. This was the basis of Fischer's proof (Fischer, 1904) that the swinging leg does not act as a pendulum. On the basis of figure 7 it is now possible

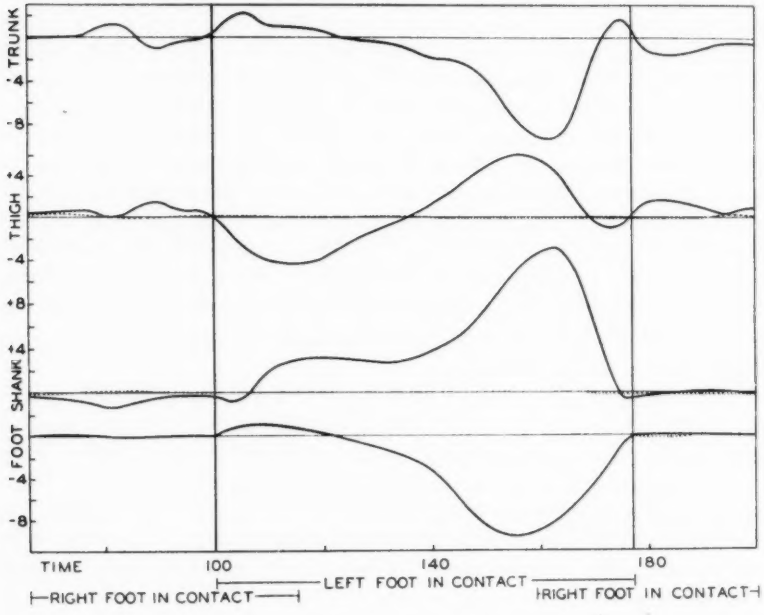


Fig. 7. Muscle and reversed effective torques in kgm. m. The muscle torques represent the resultant of all muscle torques acting on the member. Positive torques tend to produce counter-clockwise rotation. Muscle torque, continuous line; reversed effective torque, dotted.

to extend this conclusion to the body as a whole while the foot is in contact with the ground.

The tension in the muscles can be computed from the torque if the lever arms are known. In general, limiting values for tension can be obtained in this way by considering the range in values for the lever arms of various muscles which might be concerned in the production of the resultant torque. The minimum value thus calculated would be exceeded when antagonistic muscles are simultaneously under tension. The maximum torque about the ankle-joint occurred at phase 156, at which time

it was 950 kgm. cm. With a lever arm of 4.0 cm. at that moment, the triceps surae was exerting a tension of 237.5 kgm., a value 3.7 times the weight of the individual. This is by no means a maximum value for the muscle, since it occurred while walking at a very moderate rate.

*Energy transfer.* The rate at which energy is transferred, or the rate at which work is being done on or by the various components of the system, can be determined since the forces and torques and the velocities of their points of application are known. The results are plotted in figure 8 in kgm. m. / sec., from which the value in horse-power may be derived, if desired, since 1 h.p. = 76.065 kgm. m. / sec. The area under each curve represents the total energy involved. A positive area measures the amount of work done by the force on other parts of the system, a negative area the energy which it receives from the other parts.

The rates of change of potential energy and of kinetic energy due to changes in linear and angular velocity have all been added together and the negative value of this sum plotted. Consequently when the curve labelled K.E. + P.E. is positive, energy is being released from the member, either to be passed on by the joint forces or into the muscles. From the time the left heel leaves the ground (which is indicated by the resumption of muscle power on the foot), through the early part of the swing, energy is being absorbed to increase the combined kinetic and potential energy of the parts of the leg. During the latter part of the swing, shown in the first part of the graph, this energy is given back to the other parts of the system. When considered with respect to the muscle and joint forces, kinetic and potential energy changes in the leg are of more importance when the leg is swinging than when it is in contact with the ground. While the foot is on the ground it is the changes in kinetic and potential energy of the rest of the body which are of importance, as indicated by the curve for the action of the hip-joint forces on the thigh.

The rate of energy transfer due to the joint forces is plotted separately for the forces at the proximal and distal ends of each member. The work done on the member by these forces is the algebraic sum of the areas under these curves, but by plotting them separately it is possible to follow the transfer of energy from one member to those adjacent to it. The platform reaction does no work on the foot. The uppermost curve in figure 8 gives the rate at which work is done on the rest of the body by forces acting through the hip-joint.

The rate at which the muscles do work on, or receive energy from, each part of the leg is shown in figure 8 in continuous lines. This does not represent solely the energy received by the muscle in stretching or released in contraction, but also energy which is transmitted by the muscle from one point of attachment to another. The value for the power of the leg muscles acting on the trunk is obtained by multiplying the torque of these

muscles, shown in figure 7, by the angular velocity of the trunk. The determination of this torque is equal in accuracy to the determination of the other muscle torques. Difficulty, however, is experienced in measuring

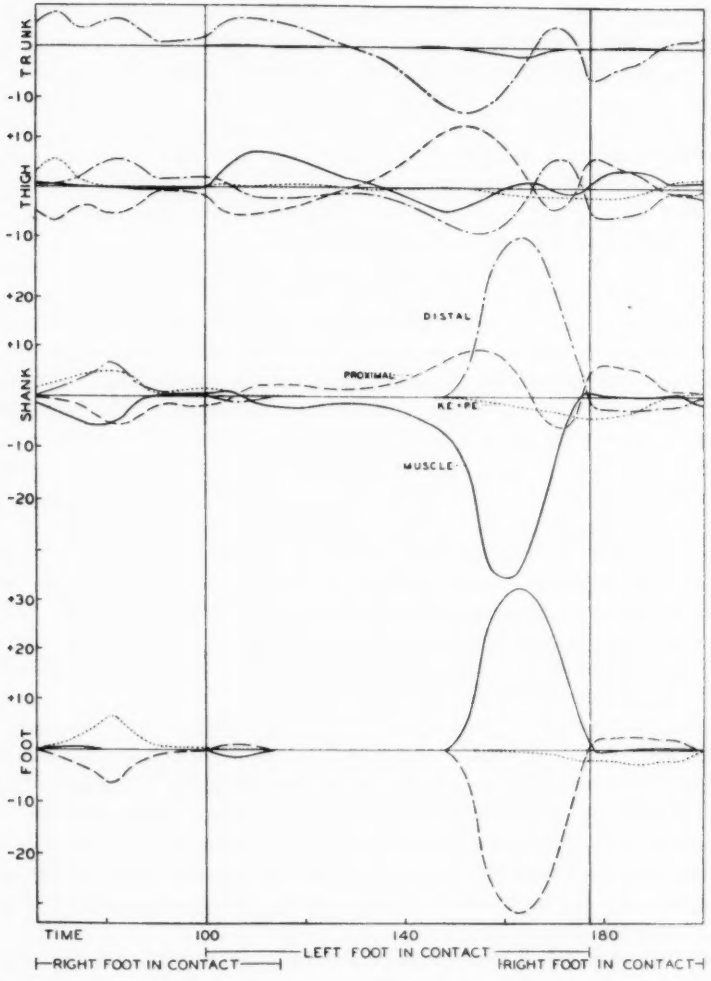


Fig. 8. Rate of doing work (power) in kgm. m./sec. Positive values indicate that energy is being expended, negative that it is being received. The area under each curve measures the total energy involved. Curves for muscle, proximal and distal joint forces, and contribution of energy from changes in combined kinetic and potential energy of member.

pelvic rotation. The values for the power plotted here agree well with those which the author has obtained from unpublished calculations based on Fischer's (1899) data.

*Transfer of energy between the leg and the rest of the body.* Before considering in detail the transfer of energy within the leg, it is advantageous to consider the leg as a whole. In figure 9 are plotted the combined values for the foot, shank and thigh of rates of energy change due to the action of muscles on the leg and of decrease in kinetic and potential energy of the leg, liberating energy for other purposes. The only joint force now present is the force at the hip-joint, acting on the leg.

In the latter part of the swing of the leg, which occupies the first portion of the graph, the leg is losing combined kinetic and potential energy, and this energy is available for other purposes. Since both the muscle and the hip-joint curves are negative at this time, the energy liberated must

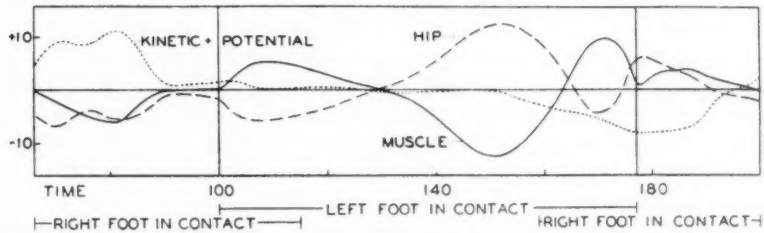


Fig. 9. Power curves for the left leg as a whole, kgm. m/sec. The muscle curve shows the rate at which muscles contribute to, or subtract from, the energy of the leg; the hip curve the rate of energy change due to joint forces at the hip; and the kinetic and potential curve the rate at which energy is contributed by the decrease in combined kinetic and potential energy of the leg.

be absorbed by the muscles and by the rest of the body, in the relative amounts indicated by the curves. The disposition of the energy received by the rest of the body cannot be traced without considering the right leg, which at this time is in contact with the ground. The energy received by the leg muscles is taken up by the muscle tissue, either for storage or dissipation. The other possibility is for these muscles to expend energy on the trunk, but the power of the leg muscles on the trunk is negligible at this time.

After the foot comes into contact with the ground, the combined kinetic and potential energy of the leg continues to liberate energy. The muscles now do work on the leg. The energy released is passed on to the rest of the body, as is indicated by the fact that the hip-joint forces are receiving energy at this time. From the time at which the right foot leaves the ground, the force acting through the hip-joint and the torque of the left

leg muscles on the trunk represent the only external forces, in addition to gravity, which act on the rest of the body. We therefore know that energy absorbed by the rest of the body must be used in increasing the sum of its kinetic and potential energies. That the potential energy of the body is increasing may be inferred from the upward displacement of the hip-joint at this time. From the velocity curves published by Fischer (1899), the velocity of the body as a whole is seen to increase as the foot comes into contact with the ground, decreasing, however, before the center of gravity of the body reaches its maximum height.

It is consequently not surprising that the hip-joint forces begin to do work on the leg, as shown in figure 9, before the hip-joint reaches its maximum elevation. This energy, at first contributed by decrease in kinetic energy of the body, later also by decrease in potential energy, is only used in small part for the increase in combined kinetic and potential energy of the leg. By far the greater part of it is received by the muscles of the leg.

During the last 0.1 sec. of contact of the foot with the ground, it is the muscles of the leg which are releasing energy. This is used chiefly in increasing the kinetic and potential energy of the leg. Some of this energy, however, is transferred to the rest of the body, although towards the end of the period of contact the rest of the body is again contributing energy to the leg.

During the early portion of the swing, the leg is increasing its combined kinetic and potential energy. This energy is contributed by the muscles and by the rest of the body, in proportions which vary as the values in figure 9 indicate.

*Transfer of energy within the leg.* During the latter portion of the swing, shown at the left in figure 8, the foot is decreasing its combined potential and kinetic energy and to this is added a small amount of work done by muscles acting on the foot. The energy thus made available is transferred to the shank by the joint forces in the ankle, since they receive energy from the foot (dashed line) and contribute it to the shank, where they constitute the distal joint forces (dash-dot line). In addition to receiving energy from the foot, the shank is also contributing energy from its combined kinetic and potential energy. Part of this energy is passed on to the thigh by the knee-joint force, the rest being received by muscles. A similar procedure is taking place in the thigh, although there the energy liberated is almost equal to that which is passed on, through the hip-joint to the trunk, leaving little energy exchange to the muscles.

After the foot makes contact with the ground, the chief source of energy is muscle action on the thigh. Part of this energy is passed on to the trunk by the hip-joint force, the remainder to the shank through the

knee-joint, although for about 0.05 sec. the knee-joint force transfers energy in the other direction. The energy carried to the shank is absorbed by muscles

From phase 130 to 150 energy is supplied by the rest of the body through the hip-joint. This energy is partially taken up by the muscles acting on the thigh, partially transferred to the shank to be received by muscles there.

From phase 150 to 165 energy is still being provided by the hip-joint force and is partially taken up by muscles acting on the thigh, except at the close of the interval, at which time the thigh is receiving a small amount of energy from the muscles. The increase in combined kinetic and potential energy of the thigh withdraws a small amount of energy. The remainder of the energy from the hip-joint force is transferred to the shank. The shank also receives a large amount of energy from the foot through the ankle-joint, this energy being released by the muscles acting on the foot. Energy is taken from the shank by the increase of its combined kinetic and potential energy, but by far the larger portion of it is received by muscles.

From phase 165 to 174 energy is being transferred to the rest of the body through the hip-joint from the thigh. Energy is also taken from the thigh by the increase in its combined kinetic and potential energy. The total amount of energy so involved is smaller than it would be if the subject had not been slowing down slightly. The chief source of the energy is by transmission from the shank. The only source of energy for the shank at this time is transmission from the foot through the ankle. Enough energy is transmitted in this way for forwarding to the thigh, for increase of the kinetic and potential energy of the shank and for reception by muscle. The source of the energy from the foot is the action of muscles on the foot.

In the first part of the swing of the leg, energy is supplied from the rest of the body through the hip-joint and from the muscles working on the thigh, this energy being transmitted so as to allow the increase in kinetic and potential energy of the shank and foot without appreciable muscular work on those members.

*Résumé of energy changes.* Starting with the leg in mid-air, we find that the combined kinetic and potential energies of the foot, shank and thigh are all decreasing, liberating energy for other purposes. Somewhat more than half of this energy is transmitted to the rest of the body, the remainder being received by the muscles, chiefly those acting on the shank.

During the first portion of the period during which the foot is in contact with the ground, the changes in kinetic and potential energy of the leg are small. Muscles acting on the thigh contribute sufficient energy for

transmission of a considerable amount to the trunk and a smaller amount to the shank, from which it is taken by muscles.

In the middle portion of the contact period, energy is given to the leg by the rest of the body, to be received by muscles acting on the thigh and shank.

When the heel starts to rise, muscles acting on the foot liberate energy, which is transferred to the shank, to be received by muscles acting on the shank. The rest of the body contributes energy to the thigh, from which it is partially taken by muscles acting on the thigh, partially transferred to the shank to be received by muscles there. The kinetic and potential energy of the leg is also increasing at this time.

Toward the conclusion of the period of contact, the rest of the body is receiving energy from the leg. This energy, together with that which is necessary for the increase in kinetic and potential energy of the leg, is supplied by muscles acting on the foot.

During the first portion of the swing, the kinetic and potential energies of all portions of the leg are increasing. The energy for this increase is supplied by the rest of the body through the hip-joint and by muscles acting on the thigh.

The reception and release of energy by the leg muscles take place in two complete cycles for each double step. The first cycle starts in the middle of the swing, the second while the foot is on the ground.

Discussion. Considered as a locomotor mechanism, the human body represents a compromise between the principles of physical efficiency and the dictates of sound anatomical structure. A wheel rolling upon a level surface need only supply enough energy to overcome friction in order to progress with a uniform velocity. Even on an undulating surface the wheel may roll on without expending extra energy, by converting kinetic energy into potential as it rises, reconverting it into kinetic as it falls. This implies changes in velocity but not of total energy.

The human mechanism overcomes the problem of external friction by not rolling, but introduces difficulties of a more serious nature. Not only does the combined center of gravity move in such a fashion that no exact conversion of kinetic into potential energy is possible, but the limbs must swing forward and backward with consequent flux of energy.

Muscles not only provide forces which guide the limbs into trajectories impossible for compound pendulums, but they also regulate the energy distribution of the body. When the total kinetic and potential energy of the mechanism increases, the energy is supplied by muscles. When the total energy decreases, it is taken up by muscles. The extent to which the energy received may again be issued is a moot question. If the muscles were perfect accumulators and were able to exert force without the expendi-

ture of chemical energy, then the efficiency of human locomotion would approach that of the rolling wheel, the friction of the joints and resistance to deformation of the tissues supplanting the friction of the ground. It is consequently not the innate mechanical structure of the body which limits locomotor efficiency as much as it is the imperfect qualification of muscle tissue for the functions it is called upon to perform.

## SUMMARY

1. By recording the reaction of the ground and its point of application, together with motion pictures of the displacement of the body, it is possible to study the kinetics of the leg without the necessity of considering the entire body.

2. The method of determining the instantaneous value of the forces and torques acting on each part of the leg is described. By this means the torques exerted by the muscles on the foot, shank and thigh, and of the leg muscles on the trunk, are found.

3. The transfer of energy within the leg and between the leg and the rest of the body is followed by means of the activity of the forces and torques.

4. The total kinetic and potential energy of the leg increases as the leg is swung forward, decreasing again from the middle of the swing until the foot is in contact. This increase and decrease in energy is only partially balanced by transfer of energy from or to the rest of the body. The maintenance of energy equilibrium is due to the action of muscles, which alternately receive the excess energy and supply the deficit.

5. The reception and release of energy by the leg muscles in walking take place in two cycles for each double step, the first starting when the leg is in the middle of its swing, the second while the foot is on the ground. The regular alternation of reception and release of energy suggests the possibility of partial storage of energy by the muscles.

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SUMMARY OF FUNDAMENTAL DATA AND METHOD OF CALCULATION. *Constants of the subject.* The total weight of the subject at the time of the experiment was 63.4 kgm. Using the proportions determined by Fischer (1906) the constants of the parts of the body were as follows:

| | *Foot* | *Shank* | *Thigh* |
|---|---|---|---|
| Weight in kgm. | 1.14 | 3.34 | 7.35 |
| Mass in kgm. slugs (gravitational units) | 0.00116 | 0.0034 | 0.0075 |
| Moment of inertia in kgm. slug cm.$^2$ | 0.046 | 0.33 | 1.19 |
| Length in cm. | 6.4 | 39.5 | 40.6 |
| Proportion of distance of center of gravity from lower joint to total length | | 0.58 | 0.56 |

The lengths of the shank and thigh are measured between joint axes; the length of the foot given here is the distance from the ankle joint to the center of gravity of the foot.

*Explanation of table 1.* The fundamental data from which the quantities discussed in this paper are calculated are given in the table for intervals of 0.1 sec. The actual computations were carried out for five times this number of phases. The x− and z− coördinates are given. The angle $\phi$ is the angle between the long axis of the member and the vertical axis. Parts of the body are indicated by subscripts: ankle joint, a; knee joint, k; hip joint, h; foot, 7; shank, 5; thigh, 3; trunk, 1. The velocities, or first derivatives with respect to time, are indicated by one dot, e.g., $\dot{x}$, and the acceleration, or second derivative by two dots, $\ddot{x}$. The accelerations of the centers of gravity are not given directly, to economize space, since they are multiplied by the mass of the member, which is constant, to give the effective force, denoted by E. The force exerted by the platform on the foot is shown in a horizontal component $R_x$ and a vertical component $(R_g + R_z)$ which includes components due to gravity and effective forces. The final entry is $x_r$, the x coördinate of the point of application of the force exerted by the platform.

*Method of calculation.* To illustrate the method of calculation, some of the computations for phase 150 will be presented. Since the joint force, as defined in the text, acting at the proximal, or upper, joint of a member must be in equilibrium with the other external forces acting on the member and the reversed effective force, we may write:

proximal joint force = −(distal force + force of gravity on member + reversed effective force of member)

For the foot the distal force is the platform reaction. The force is computed in vertical and horizontal, or z and x components, the term for gravity being present only for the vertical component. For the ankle-joint force, acting on the foot, the z component = −(70.0 −1.14 −0) = −68.86 or −68.9 kgm. The x component = −(−1.3 −0) = +1.3 kgm. The ankle joint force acting on the shank is of opposite sign to that acting on the foot, but of the same magnitude. With this force known, the formula given above may be applied to the shank. The z component of the knee-joint force, acting on the shank = −(68.86 −3.34 −0.3) = −65.22 or −65.2 kgm. and the x component = −(−1.3 −1.24) = +2.5 kgm. Similarly, the z component of the hip-joint force acting on the thigh = −(65.22 −7.35 +0.2) = −58.1 kgm. and the x component = −(−2.5 −1.3) = +3.8 kgm.

The torque of the muscles acting on the foot must be in equilibrium with the torques due to external forces and the reversed effective torque. Taking moments about the ankle joint, the lever arm of the vertical component of the platform reaction is the horizontal distance of the point of application of the force from the ankle-joint, or 131.4 −119.0 = 12.4 cm., and the torque is 70 × 12.4 = + 868 kgm. cm. The

lever arm of the horizontal component is the distance of the floor from the ankle-joint, or −8.5 cm., and the torque is −(−1.3) (−8.5) = −11 kgm. cm. The lever arms of the forces acting at the mass center of the foot are 6.4 cos 52.6° = +5.1 cm. for the vertical components and −6.4 sin 52.6° = −3.9 cm. for the horizontal component. The torque of gravity is therefore −1.1 × 5.1 = −5; of the vertical reversed effective force, 0 × 5.1 = 0; and of the horizontal reversed effective force, −0 (−3.9) = 0. The reversed effective torque is obtained from the moment of inertia and

TABLE 1

| TIME | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $x_a$ | 66.0 | 96.7 | 113.0 | 118.2 | 119.0 | 119.0 | 119.0 | 119.0 | 119.0 | 120.2 | 124.4 | 135.5 | 153.5 | 177.5 |
| $x_k$ | 84.5 | 92.8 | 100.4 | 109.2 | 116.2 | 120.1 | 122.4 | 125.0 | 129.2 | 137.6 | 149.9 | 166.3 | 184.7 | 200.9 |
| $x_h$ | 62.3 | 69.6 | 79.8 | 90.3 | 100.8 | 110.5 | 117.7 | 125.8 | 135.1 | 144.7 | 155.0 | 164.9 | 173.4 | 181.9 |
| $z_a$ | 16.2 | 11.0 | 10.3 | 9.4 | 8.6 | 8.5 | 8.5 | 8.5 | 8.5 | 10.8 | 15.0 | 20.5 | 23.7 | 18.7 |
| $z_k$ | 51.1 | 50.3 | 47.7 | 47.8 | 47.9 | 48.0 | 47.9 | 47.5 | 46.7 | 46.2 | 45.0 | 45.2 | 47.9 | 50.5 |
| $z_h$ | 85.2 | 83.6 | 82.7 | 83.7 | 85.5 | 87.5 | 88.2 | 88.1 | 86.9 | 86.2 | 85.3 | 85.8 | 86.9 | 86.4 |
| $\phi_7$ | +23.0 | +56.7 | +69.0 | +58.5 | +52.7 | +52.6 | +52.6 | +52.6 | +52.6 | +41.4 | +16.8 | −9.8 | −7.4 | +14.8 |
| $\phi_6$ | −28.0 | +5.7 | +18.6 | +13.2 | +4.0 | −1 6 | −4.9 | −8.7 | −15.0 | −26.2 | −40.2 | −51.3 | −52.2 | −36.3 |
| $\phi_3$ | +33.1 | +33.6 | +30.5 | +27.7 | +22.2 | +13.7 | +6.7 | −1.1 | −8.3 | −10.0 | −7.2 | +2.3 | +16.1 | +27.9 |
| $\phi_1$ | −6.5 | −7.1 | −7.2 | −6.7 | −5.8 | −6.1 | −7.1 | −7.7 | −7.8 | −7.3 | −6.2 | −6.2 | −6.9 | −7.5 |
| $\dot{x}_7$ | +333 | +267 | +90 | +21 | 0 | 0 | 0 | 0 | +1 | +11 | +31 | +125 | +236 | +298 |
| $\dot{x}_5$ | +201 | +154 | +91 | +64 | +27 | +17 | +14 | +18 | +33 | +67 | +109 | +171 | +204 | +190 |
| $\dot{x}_3$ | +109 | +81 | +96 | +102 | +78 | +58 | +50 | +63 | +78 | +99 | +121 | +142 | +130 | +110 |
| $\dot{z}_7$ | −74.5 | +7 5 | −12.0 | −24.0 | −4.0 | 0 | 0 | 0 | +2 0 | +22.0 | +37.5 | +61.0 | −2.0 | −73.0 |
| $\dot{z}_5$ | −29.0 | −24.0 | −10.0 | −0.2 | +0.2 | 0 | 0 | −2.0 | −1.6 | +10.6 | +19.8 | +29.7 | +21.0 | −26.0 |
| $\dot{z}_3$ | −5.9 | −23.0 | −6.9 | +10.4 | +16.0 | +5.0 | +1.0 | −5.0 | −13.4 | −9.5 | −0.3 | +13.2 | +19.0 | +2.0 |
| $\dot{\phi}_7$ | +5.20 | +4.82 | −0.30 | −1.96 | −0.57 | 0 | 0 | 0 | −0.23 | −3.40 | −5.16 | −3.50 | +2.35 | +4.90 |
| $\dot{\phi}_5$ | +5.02 | +4.25 | 0 | −1.72 | −1.18 | −0.68 | −0.55 | −0.86 | −1.36 | −2.82 | −2.02 | −1.20 | +1.20 | +4.10 |
| $\dot{\phi}_3$ | +1.28 | −0.40 | −0.27 | −0.50 | −1.86 | −1.34 | −1.28 | −1.50 | −0.93 | +0.05 | +0.80 | +1.64 | +2.84 | +1.18 |
| $\dot{\phi}_1$ | −0.15 | −0.05 | +0.01 | +0.17 | +0.11 | −0.16 | −0.14 | −0.07 | +0.03 | +0.15 | +0.15 | −0.10 | −0.14 | −0.06 |
| $\ddot{x}_a$ | +302 | +252 | +91 | +28 | +2 | 0 | 0 | 0 | +2 | +28 | +59 | +160 | +221 | +266 |
| $\ddot{x}_k$ | +127 | +73 | +91 | +92 | +46 | +27 | +22 | +34 | +53 | +95 | +145 | +179 | +192 | +138 |
| $\ddot{x}_h$ | +83 | +87 | +101 | +109 | +101 | +79 | +71 | +95 | +97 | +103 | +102 | +112 | +81 | +89 |
| $\ddot{z}_a$ | −90.0 | −18.3 | −10.0 | −15.4 | −1.1 | 0 | 0 | 0 | +3.0 | +39.0 | +50.0 | +54.0 | 0 | −79.0 |
| $\ddot{z}_k$ | +11.5 | −36.0 | −10.0 | +4.5 | +3.6 | −0.7 | −1.9 | −5.4 | −10.0 | −9.8 | −5.0 | +14.6 | +37.0 | +17.0 |
| $\ddot{z}_h$ | −19.6 | −19.0 | −4.4 | +15.0 | +29.6 | +12.2 | +4.3 | −6.4 | −15.5 | −9.2 | 0 | +12.0 | +5.0 | −6.0 |
| $\ddot{\phi}_7$ | +18.0 | −30.3 | −40.0 | +12.0 | +10.0 | 0 | 0 | 0 | −5.0 | −22.0 | −6.0 | +52.0 | +9.5 | +31.5 |
| $\ddot{\phi}_5$ | +29.0 | −38.0 | −25.4 | −3.5 | +6.3 | +5.1 | −1.0 | −4.4 | −6.5 | −7.5 | +3.5 | +17.8 | +29.4 | +23.4 |
| $\ddot{\phi}_3$ | −27.6 | −8.0 | +3.0 | −7.0 | −2.8 | +2.1 | −1.4 | +0.8 | +9.0 | +10.6 | +10.8 | +8.4 | +4.0 | −13.5 |
| $E_{x7}$ | 0 | −2.5 | −1.2 | −0.6 | 0 | 0 | 0 | 0 | 0 | +0.2 | +0.7 | +1.1 | +1.0 | +0.4 |
| $E_{x5}$ | −1.1 | −2.1 | −0.9 | −1.7 | −0.5 | −0.2 | 0 | +0.3 | +1.24 | +1.4 | +2.15 | +1.7 | +0.3 | −0.4 |
| $E_{x3}$ | −4.1 | +1.0 | +1.4 | −0.8 | −1.6 | −1.4 | +0.5 | +1.0 | +1.3 | +1.8 | +1.3 | 0 | −0.8 | −1.7 |
| $E_{z7}$ | +0.3 | +0.3 | −0.4 | +0.2 | +0.1 | 0 | 0 | 0 | 0 | +0.1 | +0.3 | −0.3 | −0.9 | +0.1 |
| $E_{z5}$ | +0.3 | −0.4 | +0.4 | +0.1 | 0 | −0.1 | −0.1 | 0 | +0.3 | +0.4 | +0.4 | +0.4 | −1.3 | −1.1 |
| $E_{z3}$ | −0.7 | −1.7 | +1.7 | +1.2 | −0.8 | −0.6 | −0.2 | −0.2 | −0.2 | +0.6 | +0.8 | +1.4 | −2.3 | −0.8 |
| $R_x$ | | | | | −8.4 | −6.1 | −2.5 | −1.5 | −1.3 | +1.6 | +7.9 | | | |
| $R_g + R_z$ | | | | | +50.0 | +68.4 | +56.0 | +55.6 | +70.0 | +76.9 | +36.0 | | | |
| $x_r$ | | | | | 118.5 | 119.6 | 121.5 | 125.4 | 131.4 | 131.9 | 133.3 | | | |

angular acceleration and is −0.046 (−5.0) = 0, since the calculation of the other torques is not carried beyond the decimal point. The sum of the torques is consequently +852 kgm. cm.; since the muscle torque must be in equilibrium with this torque, it must have a value of −852 kgm. cm.

Similar computations can then be made for the shank and the thigh, the lever arms of the forces being obtained from the positions of the joints or from the length of the member and its angle of inclination. The proportionate position of the mass center with respect to the joints allows the calculation of the lever arms of forces

acting at the mass center. When these computations are carried out, they yield a value of +755 kgm. cm. for the muscle torque on the shank and of +503 kgm. cm. on the thigh. Since the total torque exerted by any system of muscles must be zero, and the muscles of the leg end either in the leg or on the trunk, the torque which they exert on the trunk is $-(-852 + 755 + 503) = -406$ kgm. cm.

The rate at which the forces and torques are doing work, or their activity, is readily calculated since the velocities of the points of application of the forces and the angular velocities of the members upon which the torques act are known. Taking the thigh as an illustration, the activity of the muscle torque is $+503\ (-0.93) = -467$ kgm. cm./sec.; of the distal joint force, with x and z components, $(-2.5 \times 53) + (+65.2)\ (-10.0) = -785$; and of the proximal joint force, $(+3.8 \times 97) + (-58.1)\ (-15.5) = +1268$.

In similar fashion the rates at which the potential and kinetic energies of the thigh are decreasing may be obtained from the activity of the force of gravity and of the reversed effective forces and torques. For decrease in potential energy the rate is $-7.35\ (-13.4) = +99$ kgm. cm./sec.; for kinetic energy, giving the x and z components of reversed effective force and the reversed effective torque in order, the rate of decrease is $-1.3 \times 78 + 0.2\ (-13.4) + (-1.19)\ (+9.0)\ (-0.93) = -94$ kgm. cm./sec. The rate of decrease in combined kinetic and potential energy is consequently $99 - 94 = +5$ kgm. cm./sec. The computations for the other parts of the leg are made in comparable fashion.

# THE FUNCTION OF MUSCLES IN LOCOMOTION

HERBERT ELFTMAN

*From the Department of Zoology, Columbia University, New York City*

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The analysis of the forces involved and of the energy transfers which take place in walking, presented in the previous paper, serves to concentrate attention on the dominant rôle which muscles play in locomotion. Not only do the muscles provide forces which modulate the other forces present so as to ensure the desired trajectory of the body, but they also govern the fluctuations in energy, occasioned by the changes in kinetic and potential energy of the body. This involves the alternate acceptance and release of energy by the muscles of the leg, the process going through two complete cycles during each double step.

In order to obtain further information concerning the function of muscles and to investigate some of the factors which influence their efficiency, it is necessary to recast in terms of muscle units the data obtained in the form of resultant muscle torques on parts of the leg. This could be done and a unique solution obtained, if the muscles with which we are concerned in all cases passed over only one joint. Although the vasti and many of the muscles around the hip-joint satisfy this requirement, such important muscles as the hamstrings, rectus femoris and gastrocnemius traverse two joints. No unique solution is possible for such a system with the data at hand, but the limiting conditions can be found by a consideration of a system composed entirely of one-joint muscles and of one consisting of a single muscle passing over the three joints.

*Torques of one-joint muscles.* If only one-joint muscles were present, their torques would be those which are plotted in figure 1. The torque exerted on the foot by the muscle which crosses the ankle would be the total torque on the foot, previously determined. The muscle would exert an equal and opposite torque on the shank; the soleus, for instance, provides a negative torque on the foot and a positive one on the shank. Knowing the torque of the ankle muscle on the shank and the total torque on the shank, we can compute the torque of the knee muscle. The torque of the muscle which passes over the hip-joint is already known from the muscle torque on the trunk.

The one-joint muscles would be present in pairs of flexors and extensors, unless the line of action shifted over the axis of the joint. If both members

of the pair were acting simultaneously, the curve plotted would be the algebraic sum of their torques. The alternation between flexor and extensor action may be followed from figure 1. It is noticeable that the extensors are more important at the ankle and knee, the converse being true at the hip.

*Energy transfer of one-joint muscles.* Although the torques exerted by a one-joint muscle on the members to which it is attached are equal in magnitude, but of opposite sign, no such simple relationship holds for the rate at which energy is transferred between the muscle and the two members. This depends on the torque and on the angular velocity of the member. It may best be illustrated by the ankle muscle during the later portion of the period of contact of the foot with the ground. The torque of the muscle on the shank at this time is opposed to the angular velocity of the shank. In consequence work is done on the muscle, as indicated by

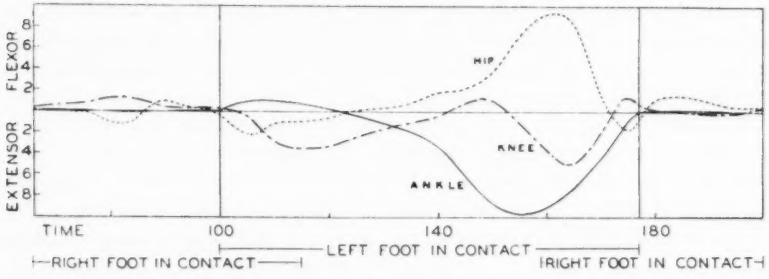


Fig. 1. Torque in kg.m. of one-joint muscles

the dashed line in figure 2. The torque on the foot is of the same sign as the angular velocity of the foot, the rate at which it is doing work being shown by the dash-dot line. The algebraic sum of these two rates represents the rate at which energy is exchanged by the muscle tissue. Energy is at first received, later work is done. The difference between the energy absorbed by the muscle tissue and the total work done by the shank on the muscle represents energy transmitted by the muscle from its origin to its insertion. This transmission may be referred to as "tendon action." It involves the presence of tension in the muscle but neither reception nor expenditure of energy by the muscle tissue, except for the production and maintenance of tension.

The uppermost curves of figure 2 give the total rate at which the leg muscle tissue does work, is worked on, and transmits energy by "tendon action." The relatively large amount of energy handled by transmission while the foot is on the ground is noteworthy. The fact that some muscles are receiving energy while others are doing work is also significant. With

muscles differently attached this antagonism of effort would be unnecessary.

The efficiency of a system of one-joint muscles is limited by the fact that tension must be maintained in at least three separate muscles at all

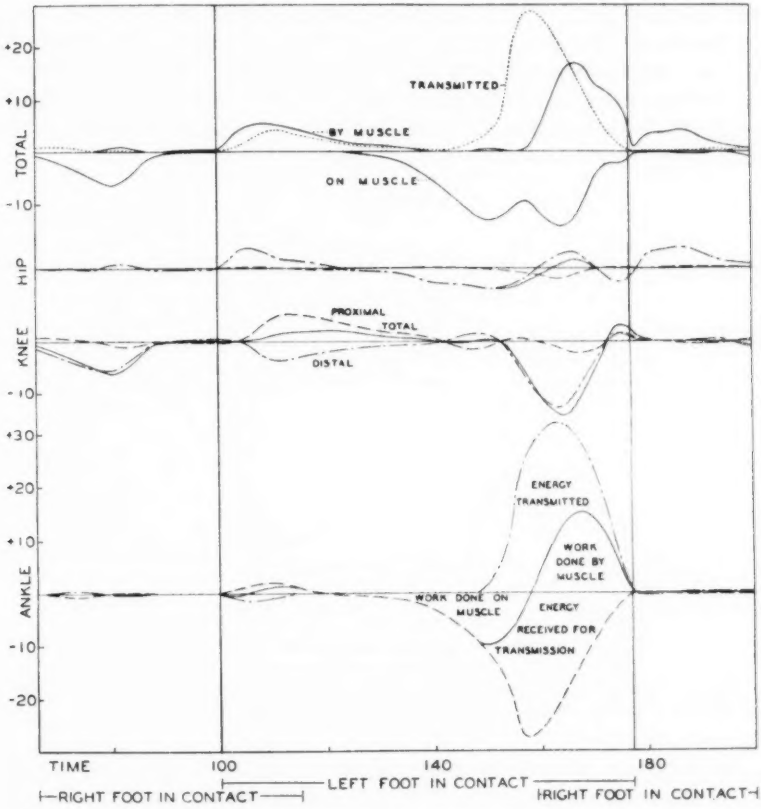


Fig. 2. Rate at which work is done by one-joint muscles in kg.m./sec. Proximal end of muscle, dashed line; distal end, dash-dot. The algebraic sum of these, giving the rate at which energy is released or received by the muscle tissue, is shown by a continuous line. Uppermost series of curves gives totals for entire leg musculature of rate of work done on muscle tissue, by muscle tissue, and transmitted.

times. The one-joint muscles do not function with a minimum amount of energy exchange, since some of the muscles receive energy while others are doing work.

*A single three-joint muscle.* From a consideration of a system of one-

joint muscles we shall go to the other extreme, discussing the situation which would be present if all the muscles traversed the hip-, knee- and ankle-joints. The characteristics of such a system may be obtained from those of the system of one-joint muscles in a manner illustrated in figure 3. The two positions of the leg chosen for illustration are phases 84 and 164 of the series. About the axis of each of the joints an arc of a circle is described, its position with respect to the leg depending on whether the muscle torque about the joint is flexor or extensor. The radii of the arcs are proportional to the torques of the one-joint muscles at the moment under consideration, the radius about the joint around which the torque is greatest being arbitrarily drawn of constant length.

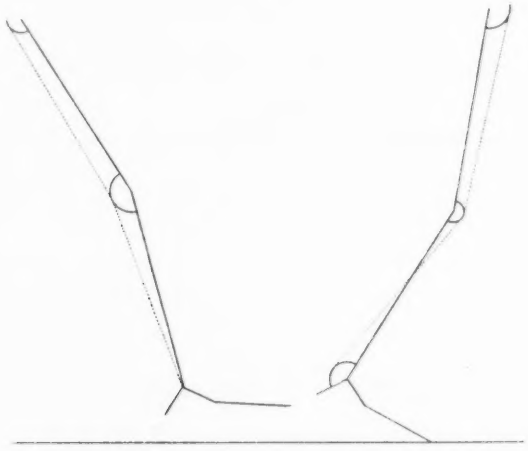

Fig. 3. Diagram of a three-joint muscle capable of performing the functions of all the leg muscles. Phase 84 at left, phase 164 at right.

The radius of the arc consequently represents the lever arm of muscles which are tangent to the arc. Since the torque of the muscle is the product of tension and lever arm, the effect obtained by increasing or decreasing the radii of the arcs, provided their relative proportions are maintained, is to decrease or increase the requisite tension in the muscle. The one-joint muscles would be represented on this scheme by lines tangent to the arcs and attached to the two adjacent members. Such a line may be tangent to the arc at only one point, the muscle then being indicated by a straight line, or the muscle may follow the arc, as if it were a frictionless pulley. The arcs may consequently be taken to represent one-joint muscles.

Any muscle which is so placed as to be tangent to the arcs described will

provide the proper torque about the joints. It is consequently possible, in theory, to have a single muscle attached only to the trunk and the foot which will provide all of the muscle torques necessary and take care of all the energy changes. Such a muscle would have the same tension throughout its length, the torques at the various joints depending on the lever arms. In the three-joint muscle illustrated, the lever arm is arbitrarily held to a constant value at the joint about which the torque is greatest, this being the knee-joint at phase 84 and the hip-joint at phase 164. The tension in the muscle would consequently vary, its instantaneous value being shown by the lowermost curve in figure 4, for a muscle with a con-

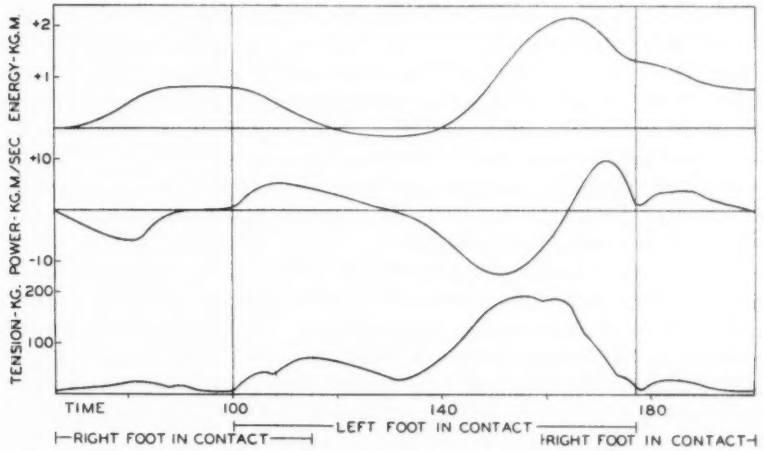


Fig. 4. Characteristics of a three-joint muscle with a constant lever arm of 5 cm. at the joint about which it produces maximum torque. Lower curve: tension in kg. Middle curve: power in kg.m./sec. Upper curve: integrated energy exchange of muscle due to external forces, in kg.m.

stant maximum lever arm of 5 cm. This value for the lever arm was chosen because it lies within the range of the maximum lever arms of the major leg muscles.

The fact should be stressed that the three-joint muscle considered here is only one of a family of such possible muscles. The disposition of the muscle is determined by the signs and relative magnitudes of the torques about the various joints. The absolute magnitudes of the lever arms and the tension in the muscle are related only by the requirement that their product shall equal the torque. In the example given, the lever arms are subjected to the condition that the maximum lever arm remains constant, the variations in tension being thus determined.

*Properties of the three-joint muscle.* It is a common property of all muscles not encountering frictional resistance in their course that the tension must be the same throughout the length of the muscle. A possible advantage of a three-joint muscle over a system of one-joint muscles is that the former would need to maintain tension in only one muscle instead of in three. The values of the tension in the muscle, given in figure 4, are for a muscle with a constant maximum lever arm of 5 cm. Since this value for the lever arm is near the maximum of the actual leg muscles, the area under the tension curve, measuring tension-time, may be considered as an approximate minimum for the muscles concerned in this particular double step.

The rate at which the muscle tissue of the three-joint muscle exchanges energy is shown by the middle curve of figure 4. This is equal to the algebraic sum of the rates of all the leg muscles, obtained by a consideration of their energy exchange with the separate parts of the leg and with the trunk, or by the analysis of one-joint muscles. It is the curve which would be obtained by adding the two curves at the top of figure 2 for the total work done on and by one-joint muscles. All of the muscle tissue is either receiving or liberating energy at the same time; different muscle masses are not working against each other.

The energy exchange goes through two cycles in the course of a double step. Energy is received during the later part of the swing and work is then done in approximately equal quantity. Energy is again received, followed by the doing of work as the heel rises and during the early portion of the swing. The time between the maximum rate of reception of energy and the maximum rate of doing work is 0.26 sec. for the first cycle and 0.20 sec. for the second cycle in this particular step.

The total energy exchange of the muscle mediated by external forces can be obtained from the power curve by integration. It is plotted in kilogram meters as the uppermost curve of figure 4. That this curve does not reach the base line at the conclusion of the double step is due to the fact that the subject slowed down slightly, as is evident from the recorded velocities. If the velocities and positions of all parts of the body were identical at the end of the step and at the beginning, except for forward movement of the entire body, the energy curve would terminate on the base line.

*Combination of two-joint and one-joint muscles.* A three-joint muscle of the type considered does not enter into the actual construction of the leg. It would be exceedingly difficult to provide, in the body, the changeable lever arms demanded. This is especially true when they swing across the axis of the limb. That such a system is not entirely preposterous, however, is indicated by muscles such as the gracilis, which exerts either a positive or a negative torque on the trunk, depending on the position of the

thigh. Another difficulty encountered by a leg containing only one three-joint muscle would be that the advantage in walking would be more than offset by the inability of such a muscle to allow individuated movements of separate parts of the leg, so necessary under other circumstances.

A compromise is effected by having a combination of two-joint and one-joint muscles (disregarding the presence of the lower ankle-joint and the transverse tarsal joint in classifying the muscles). The two-joint muscles retain many of the advantages of the three-joint muscle and, in combination with the one-joint muscles, render the system adaptable to varying requirements. Among the advantages retained in part by muscles passing over two joints are the maintenance of tension in less muscle tissue and a reduction of the necessity of having some muscles release energy while others are receiving it. There is also the possibility in muscles which pass over more than one joint of placing the muscle tissue closer to the trunk. This reduces the moment of inertia for swinging the leg about the hip-joint, while increasing it for rotation about the ankle. Since it is the forward swing about the hip-joint which occurs with the greater velocity, this arrangement is advantageous.

It is possible to ascertain which of the two-joint muscles actually present in the body would be in proper position for the exertion of torque by referring to figure 3. In the first illustration, of phase 84, the hamstrings would be properly placed, while at phase 164 the rectus femoris is correctly located. Neither of these muscles, however, has the proper ratio of lever arms about the hip- and knee-joints to exert the torques alone. At phase 84, the long head of the biceps, representing the hamstrings, has a lever arm of 6.7 cm. about the hip-joint and 3.4 cm. about the knee. It would be necessary to supplement its action by either a one-joint flexor of the knee or by a one-joint flexor of the hip. The third possibility, of concurrent action by the gastrocnemius, is acceptable for the knee but not for the ankle.

At phase 164 the rectus femoris has a lever arm of 3.9 cm. at the hip-joint and 4.4 cm. at the knee. It could be supplemented by a one-joint flexor at the hip-joint, leaving only the ankle-joint which the soleus could provide for amply. If the gastrocnemius were in action, the torque of the rectus about the knee-joint could be increased, necessitating less one-joint flexor action at the hip-joint.

These two illustrations indicate both the possibilities and the difficulties of extending the analysis which we have made of one-joint and three-joint muscle systems so as to include two-joint muscles. Such a variety of possible combinations is afforded that no unique solution can be found for such a system. Its efficiency must lie between that of the two limiting cases: a system consisting of one-joint muscles and one containing only one three-joint muscle.

As far as the exertion of torque is concerned, the intervals during which any one of the two-joint muscles present in the leg might be profitably employed may be determined from figure 1. In the later portion of the swing, which occupies the first part of the graph, the knee torque is flexor and the hip torque extensor; this condition is satisfied by the hamstrings and by the gracilis. Both torques then become flexor, which is true for the sartorius. As the foot touches the ground, the hamstrings and gracilis

| TIME | 70 | 80 | 90 | 100 | 110 | 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $L_{57}$ | +5 | −2 | 0 | +6 | +104 | +17 | −113 | −337 | −852 | −887 | −432 | +8 | +6 | +5 |
| $L_{35}$ | −55 | −138 | −39 | −19 | +295 | +330 | +166 | +44 | −97 | +369 | +221 | −16 | +11 | −29 |
| $L_{13}$ | −9 | −128 | +86 | +41 | −94 | −45 | +37 | +173 | +406 | +899 | +190 | +121 | +102 | +49 |
| $L_{57} \cdot \dot{\varnothing}_7$ | +26 | −10 | 0 | −12 | −59 | 0 | 0 | 0 | +196 | +3011 | +2230 | −28 | +14 | +26 |
| $L_{75} \cdot \dot{\varnothing}_5$ | −30 | +8 | 0 | +10 | +122 | +13 | −62 | −290 | −1162 | −2502 | −873 | +10 | −7 | −20 |
| Ankle muscle | −4 | −2 | 0 | −2 | +63 | +13 | −62 | −290 | −966 | +509 | +1357 | −18 | +7 | +6 |
| $L_{35} \cdot \dot{\varnothing}_5$ | −276 | −586 | 0 | +33 | −348 | −224 | −91 | −38 | +132 | −1040 | −446 | +19 | +13 | −119 |
| $L_{53} \cdot \dot{\varnothing}_3$ | +70 | −55 | −11 | −10 | +549 | +442 | +212 | +66 | −90 | −18 | −177 | +26 | −31 | +34 |
| Knee muscle | −206 | −641 | −11 | +23 | +201 | +218 | +121 | +28 | +42 | −1058 | −623 | +45 | −18 | −85 |
| $L_{13} \cdot \dot{\varnothing}_3$ | −11 | +51 | −23 | −20 | +175 | +61 | −47 | −260 | −377 | +42 | +152 | +199 | +289 | +58 |
| $L_{31} \cdot \dot{\varnothing}_1$ | −1 | +7 | −1 | −4 | +10 | −7 | +5 | +12 | −12 | −135 | −29 | +12 | +14 | +3 |
| Hip muscle | −12 | +58 | −24 | −24 | +185 | +54 | −42 | −248 | −389 | −93 | +123 | +211 | +303 | +61 |
| Total+ | 0 | +58 | 0 | +23 | +449 | +285 | +121 | +28 | +42 | +509 | +1480 | +256 | +310 | +67 |
| Total− | −222 | −643 | −35 | −26 | 0 | 0 | −104 | −538 | −1355 | −1151 | −623 | −18 | −18 | −85 |
| "Tendon" | 96 | 8 | 0 | 20 | 407 | 231 | 96 | 50 | 286 | 2544 | 902 | 10 | 20 | 57 |
| Integrated energy | +5.0 | +49.6 | +80.6 | +79.9 | +40.7 | −0.9 | −14.7 | +4.1 | +95.6 | +201.4 | +185.3 | +127.9 | +90.8 | +77.2 |
| Tension | 11.0 | 27.6 | 19.2 | 8.2 | 59.0 | 66.0 | 33.2 | 67.4 | 170.4 | 179.8 | 86.4 | 24.2 | 20.4 | 9.8 |

*Explanation of table.* The torque of a muscle is indicated by L, the subscripts referring to the members of the body to which it is attached, the foot being 7, the shank 5, the thigh 3 and the trunk 1. The notation $L_{57}$ consequently stands for the torque of a one-joint muscle connecting the shank and the foot, as it acts on the foot. The torque of the same muscle, as it acts on the shank would be $L_{75}$. The activity of the muscle as it acts on the foot is $L_{57} \cdot \dot{\varnothing}_7$, in which $\dot{\varnothing}_7$ is the angular velocity of the foot, given in the table accompanying the previous paper. The algebraic sum of the activities of the muscle on both members to which it is attached gives the work done by (+) or on (−) the muscle tissue. The resulting sums for the three muscles are totalled separately for the muscles that are doing work at the moment and those upon which work is being done. When a muscle is doing work upon one of the members to which it is attached and is worked upon by the other, the rate at which energy is transmitted from one member to the other by "tendon action" is given by the smaller of the two values for the activity of the muscle on the two members. These rates have been totalled for the three muscles without regard to sign.

The rate at which energy is exchanged by the three-joint muscle tissue is the algebraic sum of the rates of work done by and on the three one-joint muscles; it is not listed separately. The result of integrating this curve is given. The tension of the three-joint muscle described in the text is obtained by dividing the largest torque of a one-joint muscle for each instant by five.

would momentarily be advantageous. Almost immediately both torques become extensor and so continue for a third of the period of contact. There is no two-joint muscle which has this action. The condition could be met by a combined action of the hamstrings and the rectus femoris, since the former have their longer lever arm on the trunk and the latter on the shank. The hip-joint torque then becomes flexor while that of the knee remains extensor, affording congenial conditions for the rectus

femoris. This continues almost until the foot leaves the ground, except for a brief interval during which the knee-joint torque is flexor. During this interval both the sartorius and the gracilis, the origin of which now lies in front of the hip-joint, could act. Just before the foot leaves the ground the hamstrings are again in correct position, followed, during the swing, by the rectus femoris.

The gastrocnemius is placed for advantageous torque exertion during the brief intervals in which the knee-joint torque is flexor, while the foot is in contact with the ground. This occurs once as the foot is about to leave the ground and once about 0.2 sec. earlier.

Discussion. The present investigation has resulted in definite information concerning the rôle which muscles play in a normal activity, that of walking, and has disclosed some of the factors which are important in a consideration of muscular efficiency. Instead of estimating the work done by the muscles from considerations of changes in kinetic and potential energy of the body, the actual torques exerted by the muscles on the various segments of the leg were determined and the rate at which they did work was calculated. Muscles participate in energy exchange by receiving energy; by dissipating energy; by doing work; and by transmitting energy by "tendon action."

For a full consideration of the efficiency of muscles many factors additional to those treated in this paper would have to be included. Among these are the cost of the maintenance of tension; the extent to which the production of tension is due to external forces; the extent to which energy is required for overcoming frictional resistance and in non-elastic deformation; and the limitations placed upon muscles by the necessity of nervous coördination. The dissipation of energy by muscles emerges as a factor of double interest. Not only is it important because it limits the extent of possible energy storage; it is indispensable when the body must lose energy, as in deceleration.

## SUMMARY

1. Muscles function in locomotion by 1, exerting torques which coöperate with the other forces present in determining the movements of the body; 2, regulating energy exchange, by transmitting, absorbing, releasing and dissipating energy.

2. The exertion of torque, without movement, and the transmission of energy by "tendon action" involve the doing of no physical work by the muscle tissue. They do involve the production of tension and its maintenance.

3. The total energy received by the muscles of the leg is equal to the total amount of work which they do in the course of a double step, pro-

vided that the initial condition of the body is duplicated at the end of the step.

4. A single muscle passing over the hip-, knee- and ankle-joints would be able to perform efficiently all the functions of the leg muscles in walking. Such a muscle, however, would present structural difficulties and would not be adaptable to individuated movements. The actual system of one-joint and two-joint muscles represents a compromise between the efficiency of the three-joint muscle and the adaptability of those passing over only one joint.

5. The ability to dissipate energy, regrettable as it may seem during the maintenance of regular movement, is as necessary a function of muscle as any other, since it makes deceleration possible.

# THE STRENGTH-DURATION RELATION OF THE NORMAL, POLARIZED AND NARCOTIZED NERVE FIBER

ICHIJI TASAKI

*From the Physiological Institute, Keio University, Yotsuya, Tokyo*

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Since Lapicque (1906, 1926), Lucas (1907) and others published their historical experiments on the relation between the strength and the duration of the current necessary to excite a tissue, much work has been done by many investigators in tracing this relation in nerve or muscle. There has been, however, a wide divergence in detail of the various sets of experimental data obtained. This divergence seems to attest immediately the complexity of the excitable tissues.

A nerve, for example, is comprised of hundreds of nerve fibers with different irritability and, furthermore, these fibers are surrounded by a thick layer of connective tissue. When a constant current pulse is applied between two points on such a nerve trunk, the shape of the current pulse is known to be distorted by the polarization of non-excitable tissues (Bishop, 1928). Further, the polarization resistance of these surrounding tissues presumably changes the distribution of potential along the nerve fiber inside. It therefore seemed worth while to investigate this problem in detail by the use of *isolated single nerve fibers*, which is obviously one of the simplest forms of excitable tissues.

In previous papers (1936, 1938a) I have shown that, in the medullated nerve fiber, complete desiccation of the medullated region alone does not inflict upon the fiber any noticeable change in excitability or conductivity if all the nodes of the fiber are kept in Ringer's solution. This property of the nerve fiber was utilized first for demonstrating that narcotics affect the nerve fiber only at the nodes of Ranvier (Tasaki, 1936, 1938a). But later I noticed that the same "micro-technique" can be used with several important advantages for stimulation of the nerve fiber.

The principle of the technique consists in insulating a nerve fiber between two neighboring nodes of Ranvier by desiccating the internodal region on glass capillaries (which I call a "ridge-insulator") and immersing electrodes in each pool of Ringer on both sides of the insulated region (see figs. 2 and 3). When this type of special fluid electrodes is used, the resistance of the electrode circuit becomes always well over one megohm; hence there will obviously be no deformation of the stimulating current by

polarization at the electrodes or at the connective-tissue sheath. And, since the two pools of Ringer's solution on both sides of the ridge-insulator can be treated as being equipotential surfaces, the distribution of potential along the fiber is quite simple and known.

The experiments described in this paper were undertaken with a view to providing for the problem of electric excitation of nerve new data obtained under these extremely simple experimental conditions. The effect of narcosis and electrotonus upon the strength-duration ($V$–$t$) relation of the nerve fiber was also investigated in this connection. Further, the difference between the new data and those obtained with the usual nerve trunk is discussed with special reference to the effect of the connective-tissue sheath surrounding the nerve trunk.

METHODS. The following method of preparation of a single motor fiber is based upon the earlier methods of Adrian and Bronk (1928) and of Shimizu (see Kato, 1934). It embodies improvements introduced by Z. Kaku (unpublished) and other members of this Institute.

A nerve-muscle (e.g., sciatic-gastrocnemius) preparation of a toad or a frog is laid on the glass platform of a wooden experimental table. In the gastrocnemius, there are three (sometimes two) branches of nerve which enter the muscle from the tibial nerve. One of the branches, preferably the distal or the middle one, is left after severing the others with small scissors (fig. 1, A). Then, the blood-vessels and connective tissue around the nerve trunk are cautiously removed with needles (the region in the circle, fig. 1, A).

In the subsequent operations, only a pair of sharp (or, at some stages, blunt) needles are used for the operation. Needles, sharpened on a whetstone and fixed rigidly to the holders, can be used conveniently for cutting, teasing or separating the connective tissue or the nerve fibers. Strong pressure applied rhythmically under the tip of the sharp needle cuts these tissues more easily than a sharp scalpel.

When the cleaning is accomplished (fig. 1, B), the connective-tissue sheath which surrounds the nerve is split with sharp needles (C). In order to avoid cutting the motor fibers, it is necessary to pull the tibial nerve lightly in the direction marked by the arrows in the figure. The active fibers which run through the muscle-nerve are carefully separated with needles from the remaining inactive fibers (C and D), and this procedure is continued step by step until only the fibers connecting with the muscle are left undivided (E). The sheath is pulled out on one side and is cut across (F). The operated region is then cleaned by removing pieces of connective tissue and most of the damaged fibers. Up to this point it is possible, with practice, to operate without injuring any motor fiber, that is, without producing twitches in the muscle during operation.

The exposed nerve bundle is then split with more or less blunt needles in such a way as to leave in the middle intact nerve fibers separated distinctly from one another (fig. 2, G and H). Then the lateral strands are cut across. Care should be taken at this stage in the operation to keep the amount of Ringer's solution around the operated region as scant as possible (but not dry!), otherwise the separated fibers will group closely again and this makes the subsequent dissection quite difficult.

When the intact fibers are widely separated from one another, a small Zn-Cu couple (Kato, 1934, p. 7) is used to identify the active motor fibers, and then all the fibers are cut across except a single fiber which produces muscular twitches when

stimulated with the Zn-Cu couple (I and J). In the small nerve twig which enters the muscle, most of the large fibers are motor fibers which innervate the muscle. A rough estimation of fiber sizes with a microscope is therefore very helpful (especially for beginners) in selecting motor fibers.

In the present investigation, the sciatic-adductor magnus (caput accessorius), sciatic-gastrocnemius or sciatic-sartorius preparation of the Japanese toad (*Bufo vulgaris*) was used. When a nerve fiber with long (over 2 mm.) internodal distances was successfully isolated, the preparation was soaked in Ringer's solution for 3 to 6 hours prior to the measurement. In all cases the composition of the Ringer used was 6.5 grams NaCl, 0.14 gram KCl, 0.12 gram $CaCl_2$, 0.2 gram $NaHCO_3$, 0.01 gram $NaH_2PO_4$ and 2.0 grams glucose made to 1 liter with distilled water.

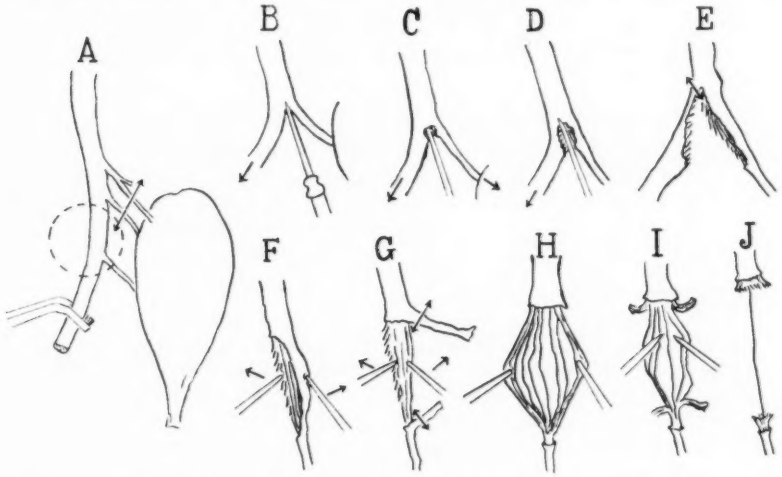


Fig. 1. Procedure of the operation to isolate a single nerve fiber from a sciatic-gastrocnemius preparation of a toad or a frog.

Then the preparation was mounted on a glass-plate with a pair of ridges ("ridge-insulator"), and the internodal region of the fiber was desiccated on the ridges (figs. 2 and 3).

A "ridge-insulator" is made of two pieces of glass capillary, both about 0.25 mm. in diameter and slightly bent, fixed tightly on a glass plate with shellac at a distance of about 0.4 mm. The upper surface of the capillary is coated smoothly with paraffin to prevent adhesion of the fluid. The space on the glass plate is filled with Ringer's solution and the preparation is floated in this pool of Ringer. A salt bridge (a glass tube filled with Ringer-agar) is placed across the ridges. The preparation in the pool of Ringer is moved under a low-power microscope so that no node of Ranvier of the nerve fiber lies between or on the ridges (fig. 2).

Then the Ringer's solution between the ridges is cautiously removed with a small

brush until the space between the ridges is completely dry. A pair of electrodes, connected together by a resistance, are immersed in the pools of Ringer, one on each side of the ridges and the salt bridge is removed. The salt bridge is to short-circuit the insulated region in order to prevent violent tetanic contractions of the muscle which are frequently caused by weak accidental currents. A preparation thus mounted on the ridges can be used for threshold determination with constant rheobase and chronaxie for over 5 hours. The electric resistance across the ridges rises, up to a few megohms at least when the fluid between the ridges is completely removed.

Non-polarizable electrodes of Zn-$ZnSO_4$-Ringer type or Ag-AgCl-Ringer type were dipped in the pools of Ringer's solution on each side of the

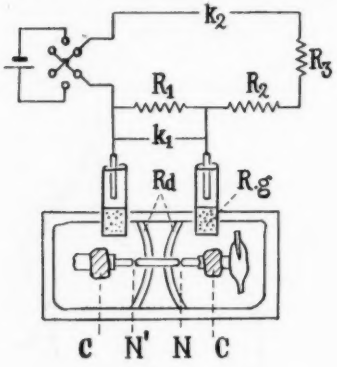


Fig. 2

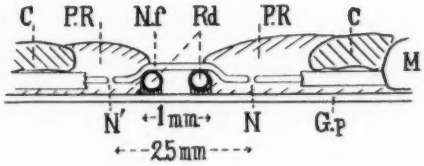


Fig. 3

Fig. 2. Arrangement used for determination of the *V-t* relation in the isolated nerve fiber. *R's:* resistances. *k's:* contacts of Helmholtz pendulum. *R.g:* Ringer-gelatin. *Rd:* ridges made of glass capillary. *N* and *N':* nodes of Ranvier. *C:* cotton wool soaked in Ringer's fluid.

Fig. 3. Diagram illustrating desiccation of the internodal region of a nerve fiber on a "ridge-insulator." *M:* muscle. *G.p:* glass plate. *P.R:* pool of Ringer. Other symbols have the same meanings as in figure 3.

ridges, and the nerve fiber was excited with rectangular current pulses supplied by the circuit shown in figure 2, top. Keys $k_1$ and $k_2$ were operated by a Helmholtz pendulum (Edelmann). One division of the scale was equivalent to 0.215 msec. The variation of the zero point of the pendulum during the course of an experiment was usually less than 0.004 msec. The resistance in the potential-divider circuit was relatively low. The variable resistances $R_1$ and $R_2$ were changed in such a way that the sum $(R_1 + R_2)$ was always equal to 2,000 ohms. The resistance $R_3$ was adjusted prior to each experiment so that a current of 0.5 milliampere flowed in the battery circuit when key $k_2$ was closed. The reading of the resistance $R_1$ therefore gave twice the strength expressed in millivolts.

A muscle twitch of strictly all-or-none character, observed by eye, was taken as the index of nerve excitation. The pause between each trial (stimulation) was held constant in one experiment and was 20 or 30 seconds in most cases. For the narcotized nerve fiber, an interval of about 10 seconds was adopted.

When the effect of electrotonus was to be investigated, the circuit shown in figure 4 was used. In the polarizing circuit, there was no knock-down key. The polarizing current was allowed to act continuously upon the nerve fiber. With this special type of fluid electrodes, the threshold was found to reach a steady level soon after application of continuous direct current.

In the investigation of the effect of narcosis, isolated nerve fibers as long as 8 mm. or more were used. At least two nodes of Ranvier were exposed in the pool of Ringer on the distal side of the ridges. The fluid in the pools (fig. 2) was replaced with a narcotizing solution (cocaine or

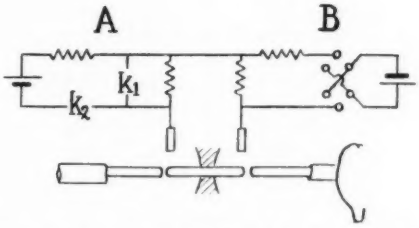


Fig. 4. Arrangement to determine the *V-t* relation in the polarized nerve fiber. A: exciting circuit. B: polarizing circuit.

urethane Ringer) and the threshold was determined. As I have pointed out (1936, 1938b; see also Kato's review, 1936), the change of irritability produced by the action of a narcotic upon the isolated nerve fiber is not progressive. A steady state is reached immediately after application of a narcotic of a given concentration. The determination of the *V–t* relation therefore could be done much more easily on single-fiber preparations than on nerve-trunk preparations.

Most of the experiments were performed during winter months, from January 1937 to February 1938. The temperature was generally between 10° and 15°C.

RESULTS. *I. Normal nerve fiber. A. Descending current.* Table 1 shows typical data of the V–t relation for descending constant current pulses. The symbol "35.5–36" in the second column of the table means that at 35.5 millivolts the stimulation was ineffective and at 36.0 millivolts, when tried after the usual pause of 30 seconds, the muscle showed a twitch.

The threshold is constant and the experiment is consistently repeatable. In favorable cases the variation in the liminal voltage for the duration of 5 msec. is less than 1 to 2 per cent for over 2 hours.

TABLE 1

*The strength-duration relation in a toad's nerve fiber (see table 4, no. 4 and fig. 1)*

| DURATION | STRENGTH ↓ | STRENGTH ↑ | $V = 12.7/t + 34.8$ |
|---|---|---|---|
| *msec.* | *millivolts* | *millivolts* | |
| ∞ | | 35.25–35.5 | 34.8 |
| 5.000 | 35.5–36.0 | 35.5 –35.75 | 37.3 |
| 0.1097 | 151 –152 | 150 –151 | 150.6 |
| 0.1634 | 112 –113 | 111 –112 | 112.5 |
| 0.2172 | 94.0–95.0 | 94.0 –95.0 | 93.3 |
| 0.3247 | 74.0–74.5 | 73.5 –74.0 | 73.9 |
| 0.4322 | 64.0–64.5 | 63.0 –63.5 | 64.2 |
| 0.5397 | 58.0–58.5 | 57.0 –57.5 | 58.3 |
| 5.000 | 36.0–36.5 | 35.5 –36.0 | |
| 0.647 | 54.0–54.5 | 54.0 –54.5 | 54.4 |
| 0.862 | 49.5–50.0 | 49.5 –50.0 | 49.6 |
| 1.077 | 46.5–47.0 | 47.0 –47.5 | 46.6 |
| 1.507 | 42.5–43.0 | 43.0 –43.5 | 43.3 |
| 2.152 | 40.5–41.0 | 42.0 –42.5 | 40.7 |
| 3.227 | 38.0–38.5 | 38.0 –38.5 | 38.7 |
| 5.000 | 36.0–36.5 | | |

TABLE 2

*The strength-duration relation in a toad's nerve fiber (see table 4, no. 7)*

| DURATION | STRENGTH ↓ | STRENGTH ↑ | $V = 18.5/t + 43.0$ |
|---|---|---|---|
| *msec.* | *millivolts* | *millivolts* | |
| ∞ | | 38.5–39.0 | 43.0 |
| 5.000 | 44.0–44.5 | 43.5–44.0 | 46.7 |
| 0.1075 | 212 –213 | 214 –215 | 215.0 |
| 0.2150 | 127.5–128.5 | 131 –132 | 129.0 |
| 0.3225 | 98.0–99.0 | 101 –102 | 100.3 |
| 0.4300 | 87.0–88.0 | 86.0–87.0 | 86.0 |
| 5.000 | 43.5–44.0 | 44.0–44.5 | |
| 0.645 | 71.0–72.0 | 72.0–72.5 | 71.7 |
| 1.075 | 60.0–60.5 | 60.0–60.5 | 60.2 |
| 2.150 | 51.0–51.5 | 51.5–52.0 | 51.6 |
| 3.225 | 47.5–48.0 | 48.0–48.5 | 49.7 |
| 5.000 | 44.0–44.5 | | |

In the last column, the values calculated by Weiss' formula

$$V = \frac{a}{t} + b$$

are given. It is evident that this old empirical formula (see Lapicque, 1926) describes the observed $V$–$t$ relation very closely. In figure 5, the

TABLE 3

*The strength-duration relation in a toad's nerve fiber (see table 4, no. 1 and fig. 9)*

| DURATION | STRENGTH ↓ | STRENGTH ↑ | $V = 11.4/t + 26.0$ |
|---|---|---|---|
| *msec.* | *millivolts* | *millivolts* | |
| ∞ | | 29.25–29.5 | 26.0 |
| 10.000 | 29.5–30.0 | 29.25–29.5 | 27.2 |
| 4.300 | 30.0–30.5 | 30.0 –30.25 | 28.7 |
| 3.010 | 30.5–31.0 | 30.5 –31.0 | 29.8 |
| 2.150 | 31.0–31.5 | 31.5 –32.0 | 31.3 |
| 1.505 | 33.5–34.0 | 33.5 –34.0 | 33.6 |
| 10.000 | 29.5–30.0 | 29.5 –30.0 | |
| 1.075 | 36.5–37.0 | 36.5 –37.0 | 36.6 |
| 0.860 | 39.5–40.0 | 39.0 –39.5 | 39.3 |
| 0.645 | 44.0–44.5 | 44.0 –44.5 | 43.7 |
| 10.000 | 29.0–29.5 | 29.5 –30.0 | |
| 0.537 | 47.0–47.5 | 47.0 –47.5 | 47.2 |
| 0.431 | 52.5–53.0 | 52.0 –52.5 | 52.5 |
| 0.322 | 61.0–61.5 | 60.0 –61.0 | 61.3 |
| 10.000 | 29.5–30.0 | 29.0 –29.5 | |
| 0.215 | 78.0–79.0 | 77.0 –78.0 | 79.0 |
| 0.161 | 96.0–97.0 | 96.0 –97.0 | 96.8 |
| 0.107 | 132.0–133.0 | 133.0 –134.0 | 132.5 |
| 0.052 | 252.5–255.0 | 250.0 –252.5 | 245.2 |
| 10.000 | 29.0–29.5 | | |

TABLE 4

*Summary of the results obtained between December 14th, 1937 and January 30th, 1938*

| EXPERIMENT NUMBER | PREPARATION (SCIATIC-) | $a/b$ | $b$ | CONTROL $t$-$V$ | TEMPERATURE | PAUSE BETWEEN STIMULI | INTERNODAL DISTANCE |
|---|---|---|---|---|---|---|---|
| | | *msec.* | *mV* | | *°C.* | *sec.* | *mm.* |
| 1 | adduc. mag. | 0.45 | 26.0 | 10–29.5 | 13.2 | ca. 10 | 2.78 |
| 2 | sartorius | 0.39 | 29.5 | 10–30 | 13.3 | 20 | 2.08 |
| 3 | adduc. mag. | 0.41 | 49.5 | 10–46 | 14.8 | 20 | 2.38 |
| 4 | sartorius | 0.37 | 34.8 | 5–36 | 8.8 | 30 | 2.05 |
| 5 | gastroc. | 0.39 | 54.6 | 5–57 | 11.1 | 30 | 2.16 |
| 6 | adduc. mag. | 0.52 | 31.0 | ∞–34 | 10.0 | 20 | 2.20 |
| 7 | gastroc. | 0.43 | 43.0 | 5–44 | 9.4 | 30 | 2.80 |
| 8 | sartorius | 0.39 | 30.5 | ∞–30.5 | 13.0 | 20 | 2.25 |
| 9 | gastroc. | 0.49 | 47.5 | 5–50 | 11.6 | 20 | 2.70 |

values derived from these data are plotted,—the voltage $V$, the quantity $Vt$ and the energy $V^2t$ against the duration $t$. The $Vt$–$t$ relation is a good straight line, and the curve $V^2t$–$t$ has a minimum at $t = a/b$.

There were, however, several cases in which the observed values of $V$ for long durations deviated from what were expected from Weiss' law. Tables 2 and 3 give such examples. In table 2, the values of $V$ for the durations over 3 msec. are consistently smaller than those given in the last column, that is to say, the coefficient $b$ in Weiss' formula is larger than the observed rheobase. In the case given in table 3, on the contrary, the coefficient $b$ is distinctly smaller than the rheobase.

Table 4 gives all of the results obtained between December, 1937 and January, 1938. In all cases, between 0.1 and 3.0 msec., the data agree well with Weiss' formula. For durations over 3.0 msec., in 5 cases (nos. 2, 4, 5, 8 and 9) the agreement is still good; in 2 cases (nos. 3 and 7) the observed values of $V$ are a little larger than the calculated ones; in the

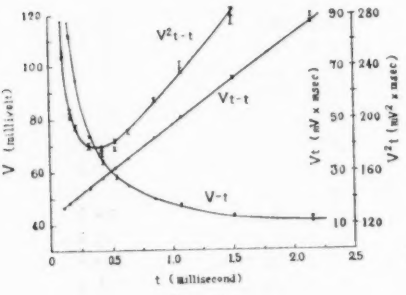


Fig. 5

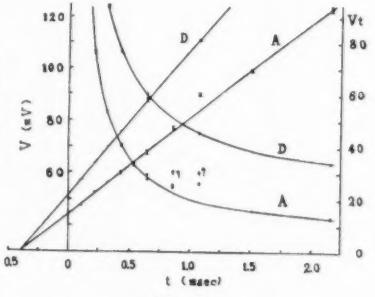


Fig. 6

Fig. 5. The $V$-$t$, the $Vt$-$t$ and the $V^2t$-$t$ relations in the normal nerve fiber drawn from the data in table 1. See also table 4, no. 4.

Fig. 6. The $V$-$t$ and $Vt$-$t$ relations obtained by ascending (A) and descending (D) current excitation. See table 4, no. 5. The point at which the $Vt$-$t$ lines cross the abscissa indicates the theoretical chronaxie (Wago and Wakabayashi, 1936), namely, the ratio $a/b$ of the coefficients in Weiss' formula.

remaining 2 cases (nos. 1 and 6) the strength is approximately constant for long durations.

*B. Ascending current.* Figure 6A shows the $V$–$t$ and $Vt$–$t$ relations. The V–t relation can best be represented by an equilateral hyperbola. The time constant $a/b$ is approximately equal to those obtained with descending current pulses.

In ascending current stimulation, the threshold is sometimes quite unstable at certain durations and deviates markedly from what is expected from the remaining part of the $V$–$t$ curve by interpolation. In figure 6, this is seen at 0.86 and 1.075 msec. This phenomenon is probably due to anodal depression at the node of Ranvier on the distal side of the ridges (N in fig. 2). For strengths over the values expected from the $V$–$t$ curve, impulses should be set up at the central node (N′ in fig. 2), but these im-

pulses seem to be blocked at the distal node before they reach the muscle. It is interesting that this block happens at only a part of the $V$–$t$ curve.

*II. Polarized nerve fiber.* Figure 7 shows the result obtained with descending current pulses during electrotonus. The nerve fiber was first cathodally polarized with a constant potential-difference of 10 millivolts, and test current pulses were superimposed upon this polarizing current. Then the polarizing current was discontinued and the $V$–$t$ relation of the unpolarized nerve fiber was determined. Lastly the direction of the polarizing current was reversed and the effect of anelectrotonus was investigated.

The $V$–$t$ relation is still an equilateral hyperbola and, as many investigators have already shown, the chronaxie (or $a/b$) is shortened by anelectrotonus and lengthened by catelectrotonus.

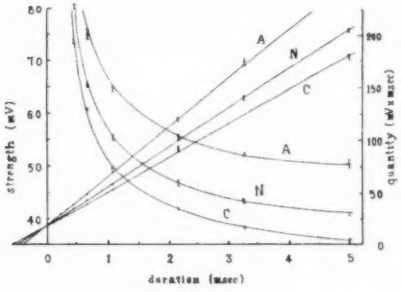


Fig. 7

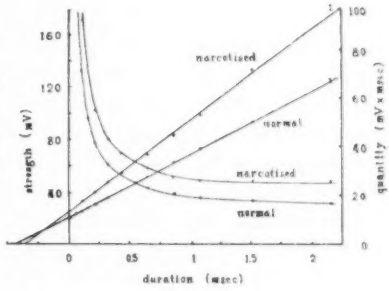


Fig. 8

Fig. 7. Strength-duration relations in the polarized nerve fiber. C: catelectrotonus. *N:* normal. *A:* anelectrotonus. Sciatic-gastrocnemius preparation. Internodal distance, 2.69 mm. Pause between stimuli, about 25 seconds. Temperature, 9.8°C.

Fig. 8. Strength-duration relation in the narcotized nerve fiber. Same preparation as used for the experiment of table 3. The normal $V$-$t$ curve is derived from table 3. A 0.5 per cent urethane-Ringer solution was used. See table 4, no. 1.

*III. Narcotized nerve fiber.* It has been shown (Tasaki, 1938b), with single nerve fibers that the rheobase and the chronaxie remain at an approximately constant level during the action of a narcotizing solution of constant concentration and do not change progressively as in a nerve trunk. The voltage rises and the chronaxie falls promptly as soon as a narcotizing solution (below the critical concentration) is applied to the isolated region of the nerve fiber and then at once an approximately steady state is reached. The value of the rheobase or the chronaxie depends solely upon the concentration of narcotic employed. It is therefore possible to determine the $V$–$t$ relation of a narcotized nerve fiber in a steady state.

Figure 8 shows the effect of narcosis upon the $V$–$t$ relation. It is clear

that the voltage rises and the chronaxie shortens in narcosis and that Weiss's formula is still applicable to the $V$–$t$ relation. This experiment is difficult, because during narcosis the irritability is less stable than in the normal nerve fiber.

As the concentration of the narcotic is increased, the change in the rheobase and the chronaxie becomes greater. The following example will give a rough estimate of this concentration-effect. In the normal state (sciatic-sartorius preparation, 13°C.) the rheobase was 38.5 millivolts and the chronaxie was 0.42 msec. These values were changed by the action of an 0.005-per-cent cocaine-Ringer solution to about 70.5 millivolts and 0.35 msec. When the concentration was increased up to 0.01 per cent, the rheobase rose to 80 millivolts and the chronaxie fell to 0.33 msec. At 0.02 per cent, however, the chronaxie was lengthened to about 0.45 msec. ("abnormal prolongation"), while the rheobase rose further to 105 millivolts. By further (but slight) increase of concentration (0.025 per cent), the nerve fiber was rendered immediately incapable of conducting an impulse through the narcotized region.

The abnormal prolongation of the chronaxie by narcosis appears only near the critical concentration (that is, the minimal concentration in which the isolated nerve fiber loses its conductivity). If three or more nodes of Ranvier are exposed in the narcotized region, this seldom occurs. An explanation of this phenomenon will be presented elsewhere.

In the narcotized nerve fiber, the anodal depression is more marked than in the normal; therefore it is almost impossible to determine the $V$–$t$ relation with ascending current pulses. It is apparent that, when the narcotized region of a nerve trunk is electrically excited, the nerve impulse is often conducted only in a single direction.

The change in the threshold brought about by the action of a narcotic is much less marked in the isolated nerve fiber than in the nerve trunk. Especially when brief current-pulses (or induction shocks) are used, the rise of the threshold is slight. The remarkable change in the threshold in the narcosis of a nerve trunk should therefore be ascribed mainly to the increase in the electric resistance of the non-excitable tissues which surround the nerve fibers.

*IV. Effect of connective-tissue sheath.* Bishop (1928) and Grundfest (1932) have shown that the $V$–$t$ relation, and with it the chronaxie, varies with the amount of connective tissue which separates the electrode and the nerve fiber. Figure 9 shows an example of similar results. First, the intact nerve trunk of the preparation was stimulated with a set of non-polarizable electrodes, and the relation (I) in the figure was obtained. Then, the connective-tissue sheath at the stimulated region was carefully removed by the method illustrated in figure 1. The nerve trunk was mounted on the electrodes as before, and the $V$–$t$ relation was determined

(curve II). Next the connective tissue inside the sheath was further torn by teasing and separating the nerve fibers from one another. Determination of the $V$–$t$ relation gave the curve (III) in figure 9.

In this type of experiment, the same spot of one and the same nerve fiber is brought into play in all cases. It is therefore obvious that not only the rheobase but also the chronaxie is markedly reduced by removal of the connective-tissue sheath and further by separation of the connective tissue inside the sheath. There are apparently two possible explanations of this effect: one is the change in the degree of distortion of the stimulating current by polarization of the connective tissue; the other is the change in the distribution of potential along the active nerve fiber by removal of the connective tissue. The present research does not indicate which one is most important. In a later paper, however, I will show that the latter effect is of great significance in the explanation of Bishop's, Grundfest's and my own experimental results.

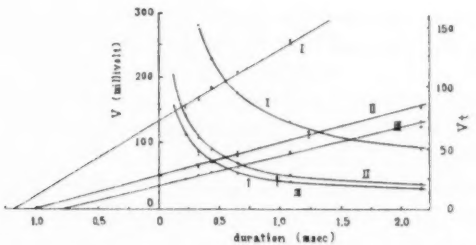


Fig. 9. Strength-duration relations obtained before (I) and after (II and III) removal of the connective tissue sheath. Sciatic-adductor magnus preparation. Electrodes: Zn-$ZnSO_4$-Ringer (gelatin) type, diameter 5 mm., interpolar distance 5 mm. Arrows mark the chronaxie. Rheobase: in (I), 59 mV; in (II), 27.5 mV; in (III), 25 mV. Temperature, 8.7°C. Pause between stimuli, about 10 seconds.

Discussion. The greatest advantage of the new method of stimulating a nerve fiber on a "ridge-insulator" is that the electrode circuit is not short-circuited by the conducting medium which surrounds the nerve fiber. Each pool of Ringer's solution on both sides of the insulated region can be treated as being always equipotential. There is no potential difference along the fiber except across the insulating ridges. Therefore, the drop of potential across the insulated region, which obviously acts upon the nerve fiber, is equal to the applied voltage.

The objection may be raised to this method, however, that the injury potential of the dissected nerve fibers may produce an appreciable effect upon the isolated nerve fiber. Such an effect, if it exists at all, would be equivalent to that of cat- or anelectrotonus caused by a weak constant current. It can be diminished by isolating as long a section of the nerve

fiber as possible and leaving the preparation in Ringer's solution for a few hours prior to experimentation. The approximate equality of the observed chronaxie for descending and ascending current pulses shows that such an effect is insignificant.

Much work has been done in the past on constant-current stimulation of the nerve, but none of it is free from the effect of non-excitable tissues which surround the nerve fiber under investigation. In the present experiments we are dealing with isolated single nerve fibers. The result obtained, therefore, in one sense, gives the standard $V$–$t$ relation in the nerve fiber. Since, however, it is shown that it is possible that the $V$–$t$ relation varies with the change of distribution of potential along the nerve fiber, it would be absurd to speak of the true or ultimate $V$–$t$ relation.

## SUMMARY

1. A modification of Shimizu and Kaku's method for isolating a single nerve fiber is described.

2. A method is described for stimulating an isolated myelinated fiber by insulating the internode on a "ridge-insulator," and the superiority of this method over the usual method is stressed.

3. The strength-duration relations were determined by the new method on the normal, narcotized and polarized nerve fiber of the toad. All the results were found to be in best accord with Weiss's empirical formula.

4. In the normal nerve fiber (at 10–15°C.), the chronaxie was found to be 0.3–0.5 msec., and the rheobasic voltage was 25–50 millivolts.

5. Sometimes with ascending current pulses, the threshold cannot be determined, due probably to block of the descending nerve impulse by the anodal depression. This occurs more frequently in the narcotized nerve fiber; the preparation sometimes gives no response for all pulse durations over a fraction of a millisecond.

6. At the same point on the nerve trunk of a motor-unit preparation, the strength-duration relation was determined before and after removal of the connective-tissue sheath. Confirming Bishop's previous result, the chronaxie in the nerve devoid of the sheath was found to be about half as long as that in the intact nerve. This effect of the connective-tissue sheath was inferred to be due to the decrease in the gradient of potential along the nerve fiber inside.

It is my wish to express to Prof. G. Kato my appreciation of his continual encouragement. To Dr. H. Davis my thanks are due for his kindness in improving the English.

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# ELECTRIC STIMULATION AND THE EXCITATORY PROCESS IN THE NERVE FIBER

ICHIJI TASAKI

*From the Physiological Institute, Keio University, Yotsuya, Tokyo*

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In various experiments on the single nerve fiber, we find that an isolated nerve fiber behaves in a grossly different manner from an ordinary nerve trunk in many respects. Sometimes we received the impression that we were dealing with an entirely new excitable tissue that had never been studied before. Especially in studying the action of narcotics, the distinction between the nerve fiber and the nerve trunk is striking. When a narcotizing solution of an appreciable concentration is applied to these tissues, the nerve fiber loses its conductivity almost instantaneously at the moment of application (Tasaki, 1936), while the nerve trunk continues to conduct an impulse for many minutes. Further, narcotics affect the conductivity, in the nerve fiber, only at the nodes of Ranvier (Tasaki, 1936 and 1938).

Kubo and Ono's experiment (1934), which revealed remarkable variation in the threshold strength along the nerve fiber, has led me, through mathematical computations, to the conclusion that the myelin sheath must be an electric insulator which covers the axis-cylinder perfectly except at the nodes (1934). This conclusion is quite consistent with the results obtained by Erlanger and Blair (1934).

The present investigation was first designed to substantiate, by applying the method of multipolar stimulation (Rushton, 1928) to the isolated single nerve fiber, my previous conclusion that the nerve impulse was set up only at the nodes of Ranvier. The range of observation was then extended to include the problems of the strength-duration relation and of the excitatory process in the nerve fiber. Though I have succeeded in confirming my previous conclusion, the results obtained in this investigation were again very different from what might be expected from our previous knowledge of the nerve trunk. Further, it appears that the strength-duration relation, and with it the chronaxie, varies with the distribution of potential along the nerve fiber. The phenomenon first observed by Davis (1923) that the chronaxie varies with the size of the electrode is explained by new experimental data.

METHODS. In all experiments, the motor nerve fibers of the Japanese

toad were used. The procedure of isolation of single nerve fibers and the details of the treatment of the preparation prior to the experimentation are essentially similar to those described in a former paper (Tasaki, 1939). Fibers with long (over 2 mm.) and approximately constant internodal distances were selected. The preparation was mounted in the pools of Ringer's solution on a glass-plate with two sets of "ridge-insulators" (fig. 1, A and B). A node of Ranvier was introduced in the middle pool between A and B and was kept in the fluid. The width of the middle pool was 1.0 mm. and the length of the desiccated regions of the nerve

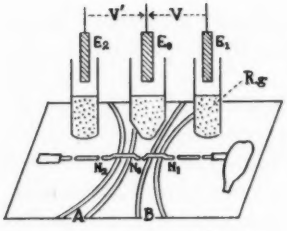
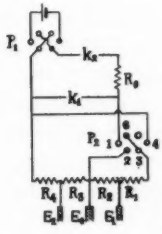



Fig. 1



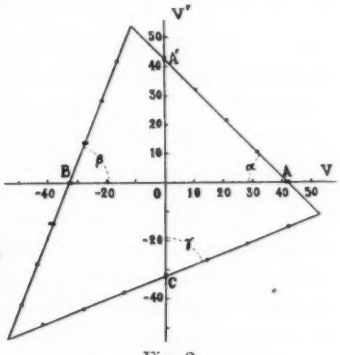




Fig. 2

Fig. 1. Tripolar arrangement used for stimulation of an isolated single nerve fiber. *E*'s: electrodes. *N*'s: nodes of Ranvier. *P*'s: Pohl's reversers. *k*'s: contacts of Helmholtz pendulum. *R*'s: resistances. *R.g.*: Ringer-gelatin. *A* or *B*: ridge-insulator made with pieces of glass capillary coated with melted paraffin. The medullated regions of the nerve fiber which lie on the ridge-insulators *A* and *B* are desiccated in the air. The remaining parts of the nerve fiber are kept in Ringer's fluid.

Fig. 2. A "triangle" obtained by tripolar stimulation of a single nerve fiber. Arrangement shown in figure 1 is used. Duration of current pulses, 5 msec. Toad's sciatic-gastrocnemius preparation. Internodal distance, both 2.20 mm. Temperature, 9.3°C. Pause between stimuli, 15 seconds. Thresholds are expressed in millivolts.

fiber was 0.8–1.0 mm. In each of these three pools of Ringer which are insulated electrically from each other, non-polarizable electrodes (Zn-$ZnSO_4$-Ringer or Ag-AgCl-Ringer) were dipped.

The arrangement of the exciting circuit is also shown in figure 1. The object of this arrangement is to produce any required potential drops, positive or negative, between electrodes $E_1$ and $E_0$ and between $E_2$ and $E_0$, independently. The resistance $R_0$ was made constant and was 1,000 ohms throughout; $R_1$, $R_2$, $R_3$ and $R_4$ were so changed that they always satisfied the relation

$$R_1 + R_2 = R_3 + R_4 = R_0 = 1{,}000 \text{ ohms}.$$

If, in Pohl's reverser $P_2$, connections are made between 1 and 2 and between 3 and 4, the potential differences $V$ and $V'$ are given by

$$V = \pm eR_2/3{,}000 \text{ and } V' = \pm eR_3/3{,}000,$$

where $e$ is the electromotive force of the accumulator (about 2.1 volts) and the symbol $\pm$ indicates the direction of the drop of potential determined by the first reverser $P_1$ (taking the directions marked by the arrows in the figure as being positive). If, on the contrary, 1 and 6 of the reverser $P_2$ are connected together,

$$V = \mp eR_2/3{,}000 \text{ and } V' = \pm eR_3/3{,}000;$$

that is, the direction of V is reversed. Thus, by the use of these two reversers any combination of V and V′, positive or negative, can be obtained.

The contacts $k_1$ and $k_2$ are operated by a Helmholtz pendulum (Edelmann); with $k_2$ closed, the opening of $k_1$ initiates the current pulse, and the subsequent opening of $k_2$ ends the pulse.

As the resistance across the ridge-insulator (A and B) is always enormously great, each pool of Ringer's solution can be treated as being equipotential. This is clear also from the experimental fact that the threshold strength is independent of the position at which the electrode $E_0$ is dipped in the middle pool. The values of V and V′, therefore, as emphatically stated in a former paper, are equal to the potential differences between the pools. There is no appreciable polarization at the electrodes, as the currents through the electrode circuits are always extremely weak.

Determinations were made by giving a series of arbitrary potential differences across one set of the ridge-insulators, and by finding for each of these what potential difference had to be given across the other set of the ridge-insulators to obtain a threshold contraction. It will be noticed that the arrangements and the procedure are quite similar to those of Rushton's quadripolar stimulation (1928) except for the smallness of the material.

Results. Figure 2 shows a typical set of experimental data obtained with current pulses of a duration of 5 msec. In this figure the potential difference V′ across the central set of the ridge-insulators is plotted as ordinate against the potential difference V across the distal set of the ridges as abscissa. The strengths are adjusted to constitute threshold stimuli. The experiment is very simple to perform and the results are consistently repeatable.

It is clear that the results lie on three straight lines which constitute a "triangle." This triangle is apparently comparable to that obtained by Rushton (1928) with his tripolar electrodes on a nerve trunk, except that the angles $\beta$ and $\gamma$ are far less than right angles.

I. *The straight line in the first quadrant and the strength-duration relation.* The straight line in the first quadrant is always almost symmetrical with respect to both axes, that is, the angle $\alpha$ is approximately equal to 45°. The greatest deviations observed are 43° (fig. 3) and 48°. As will be discussed in detail later, this line in the first quadrant corresponds to excitation at the node $N_0$ in the middle pool.

As the duration of the current is reduced, the voltage necessary to excite naturally increases. But it should be noted that the angle ($\alpha$) which the straight line makes with the abscissa is scarcely altered by changing the pulse duration in one and the same preparation. Figure 3 shows a

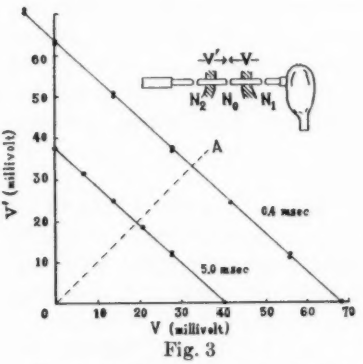



Fig. 3

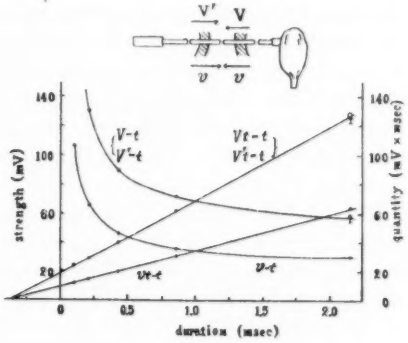



Fig. 4

Fig. 3. Straight lines in the first quadrant. Toad's sciatic-gastrocnemius preparation. Internodal distances, 2.04 mm. (between $N_2$ and $N_0$) and 2.19 mm. (between $N_0$ and $N_1$). Temperature, 12.8°C.

Fig. 4. Comparison of the strength-duration curve obtained by tripolar stimulation ($v$-$t$) with those obtained by usual bipolar stimulation ($V$-$t$ or $V'$-$t$). Toad's sciatic-gastrocnemius. Internodal distances: central, 2.65 mm.; distal, 2.98 mm. Temperature, 11.2°C. The point at which the $Vt$-$t$ line crosses the abscissa represents the theoretical chronaxie, i.e., the ratio $a/b$ in Weiss's formula.

result in which the durations of the current pulses employed were 5.0 and 0.4 msec. respectively. The two lines are parallel, the angle which they make with the abscissa being 43°.

The constancy of the slope of these lines immediately attests to the equality of the time-constants of the strength-duration curves measured with ascending current V and with descending current V′. The observed points on the horizontal axis (fig. 3) represent the liminal voltages necessary to excite the fiber with ascending current pulses of corresponding durations. If the strength-duration curve obtained with such ascending current pulses be denoted by $V = f(t)$, the curve obtained with descending current pulses V′ must be given by $V' = A\,f(t)$, where $A$ is a constant rep-

resenting tan $\alpha$. In other words, the rheobase of the $V'$–t relation may differ slightly from that of the $V$–$t$ relation but the chronaxie is always the same.

It follows from simple geometrical considerations that the same can be said of the strength-duration relation which can be obtained when the nerve fiber is stimulated with a combination of both ascending and decending currents (V and V') applied concurrently at a given constant ratio. Suppose, for example, that the middle pool of Ringer is connected to the cathode and both the central and the distal pools to the common anode (fig. 6, A), and that the strength-duration relation is determined with such arrangements. Then the potential difference $V$ is always equal to $V'$, i.e. $V:V' = 1$; this corresponds to determination of the points at which the straight line OA (which bisects the right angle at the origin) in figure 3 intersects the parallel straight lines relating $V$ and $V'$. If, therefore, the strength-duration relation for $V$ (when $V' = 0$) is denoted by $V = f(t)$ as before, the relation measured with these tripolar arrangements will be given by $v = a\,f(t)$, where $a$ is a constant which, if $\alpha = 45°$, equals $\frac{1}{2}$.

This is clearly shown in the experiment illustrated in figure 4, in which the relations $V = f(t)$, $V' = A\,f(t)$ and $v = a\,f(t)$ were determined directly on the same preparation. The arrangement shown in figure 1 was used. At varying durations between 0.1 and 2.1 msec., the values of $V$ necessary to excite when $V' = 0$, the values of $V'$ when $V = 0$ and the values of $v = V = V'$ were determined in this sequence. In this example the voltage $V$ (when $V' = 0$) was equal to the corresponding value of $V'$, that is, the angle $\alpha$ was 45°. The observed strength-duration relations were of the usual hyperbolic form and could roughly be expressed by the following formulae respectively:

$$V = 50\,(3.4/t + 1),$$

$$V' = 50\,(3.4/t + 1) \quad \text{and}$$

$$v = 25\,(3.4/t + 1).$$

The rheobase (theoretical) of the $v$–$t$ relation was just one-half of that of the $V$–$t$ or $V'$–$t$ relation, but the chronaxies (theoretical) were all the same. This is exactly what was expected from the above-stated consideration of parallelism of the straight lines in the first quadrant.

These experimental results seemed at first to indicate that every spot (every node of Ranvier) on the nerve fiber has its own natural chronaxie which is independent of the method of its determination (i.e., Lapicque's protoplasmic chronaxie, 1935). But the following investigations rendered this view untenable.

II. *The straight lines in the second and third quadrants and the strength-*

*duration relation.* In the second quadrant the direction of the potential drops V and V′ are both descending; in the third quadrant V is descending and V′ is ascending. Figure 5 gives the results of determinations made with current pulses of varying durations. Considering the wide range of the observations, all the results are seen to lie upon straight lines with great accuracy. As will be discussed later, these lines correspond to excitation at the node $N_1$ in the figure. The broken lines near the vertical axis are of course continuations of the lines in the first quadrant which correspond to excitation at the middle node $N_0$.

The most remarkable point in this experiment is the non-parallelism between these straight lines for different durations. As the duration is reduced, the angle $\beta$ which the straight line makes with the abscissa be-

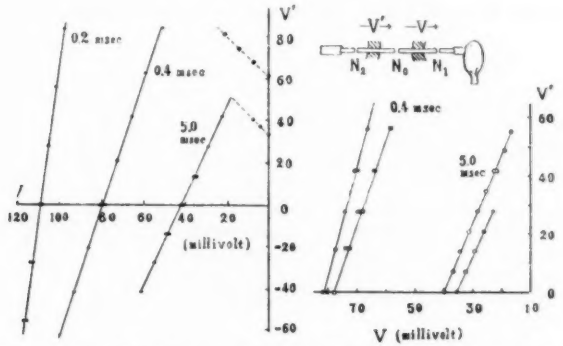



Fig. 5. Straight lines in the second and third quadrants. Left: sciatic-gastrocnemius; internodal distances, $N_1 - N_0 = 2.98$ mm., $N_0 - N_2 = 2.26$ mm.; temperature, 12.8°C. Right, circles: sciatic-sartorius; $N_1 - N_0 = 2.48$ mm., $N_0 - N_2 = 3.45$ mm.; 9.8°C. Right, squares: sciatic-gastrocnemius; $N_1 - N_0 = 2.20$ mm., $N_0 - N_2 = 2.30$ mm.

comes greater. With current pulses of a duration of 5 msec., cot $\beta$ is in most cases between 0.45 and 0.35, and, as the duration is reduced infinitely (other experiments), cot $\beta$ approaches a value between 0.25 and 0.15.

We shall again consider the strength-duration relation which can be obtained when potential differences $V$ and $V'$ are applied concurrently at a constant ratio (e.g., by using the arrangement shown in fig. 6, B). This means the determination of the points at which a straight line which passes through the origin (OA or OA′ in fig. 7) intersects the lines relating $V$ and $V'$ at different durations.

In the diagram shown in figure 7, suppose that the two straight lines represent the relations between the potential differences $V$ and $V'$ when

the pulse durations are $t$ and infinity respectively, and that the line OA in the figure represents the ratio $V:V'$ at which these two current pulses are superimposed. Then it can easily be shown that the chronaxie which can be obtained with the descending current V (when $V' = 0$) is shorter than that which may be obtained with this type of superimposed current pulses.

The voltage $V_0$ in the figure represents the rheobasic voltage measured with the simple descending current pulses, as it is nothing but the liminal voltage for infinite duration. Similarly, the rheobasic voltage measured with the superimposed current pulses is given by $(v_0 + v_0')$ which is directly proportional to $v_0$ (cf. fig. 6, B). If we take $V$ as being equal to $2V_0$, the pulse duration $t$ represents the chronaxie which can be obtained with the simple descending current pulses. Since $\beta$ in the figure is by experiment larger than $\beta_0$, $v$ must be larger than $2v_0$. Thus $(v + v')$ is larger

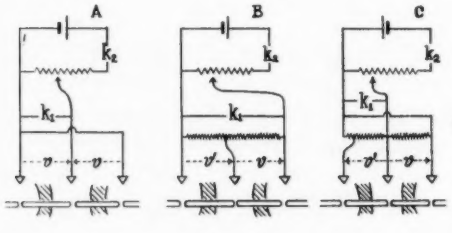



Fig. 6

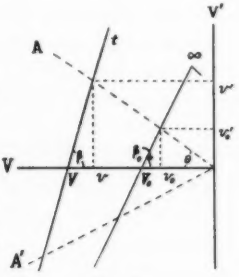



Fig. 7

Fig. 6. See text
Fig. 7. See text

than 2 $(v_0 + v_0')$, namely, more than twice the rheobase measured with the superimposed current pulses. In other words, $t$, the chronaxie measured with the simple descending current pulses, is shorter than the chronaxie which can be obtained with this type of superimposed current pulses. Thus it is clear that the chronaxie observed is longer when a nerve fiber is excited with the superimposed current pulses (V + V') than when it is excited with potential differences localized between two neighboring nodes of Ranvier.

Figure 8 gives the results of an experiment in which two strength-duration relations were alternately determined on one and the same preparation, one measured with potential differences V (when $V' = 0$) and the other determined with superimposed current pulses $v$ when $V':V = 1$. The value of the rheobase and the chronaxie were, for the former relation, 38 millivolts and 0.51 msec. and, for the latter, approximately 27.5 millivolts and 0.58 msec. respectively. The difference in the chronaxie was

about 10 per cent, but it is clear that this could be made much greater by increasing the ratio $V' : V$.

If, on the contrary, the direction of the potential drop V′ is reversed (e.g., by using the arrangement shown in fig. 6, C) so that the straight line representing the ratio $V' : V$ lies in the third quadrant (as OA′ in fig. 7), the chronaxie is shortened and the rheobase is raised. The degree of change in the chronaxie depends of course upon the ratio at which $V$ and $V'$ are applied concurrently. The greater the ratio $V' : V$, the longer is the chronaxie. It can *vary continuously* in either direction, smaller or larger, according to the sign of the potential drop $V'$.

An analytical consideration of the problem will make this point still clearer. Let the ratio $V' : V$ be $r = \tan \theta$, where $\theta$ is a constant which can vary according to the experimental condition from about 68° (or still

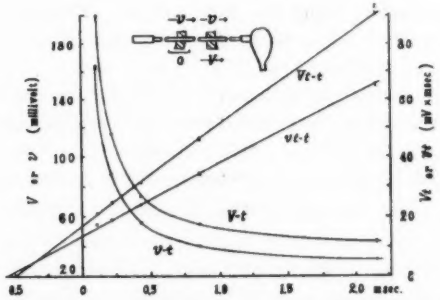



Fig. 8. Comparison of the strength-duration curve obtained by tripolar stimulation ($v$-$t$) with that obtained by bipolar stimulation ($V$-$t$). Deviation of the $v$-$t$ relation from Weiss's law is only slight in this experiment. Sciatic-gastrocnemius preparation. Internodal distances: central, 2.12 mm.; distal, 2.67 mm. 11.0°C.

more on some particular occasions) to −45° (see fig. 7). Then, for any duration of current,

$$v \tan \theta = (V - v) \tan \beta.$$

Hence

$$v = \frac{\tan \beta}{\tan \beta + \tan \theta} V.$$

Here, let the function $B(t)$ represent cot $\beta$, and let $V = f(a, b, c, \ldots, t)$ represent the strength-duration curve measured with the simple descending current pulses V, where $a$, $b$, $c$, ... are the constants which characterize the curve. Then we have

$$v = \frac{1}{1 + rB(t)} f(a, b, c, \cdots, t).$$

Denoting the right-hand side of this equation simply by $F(t)$, $v = F(t)$ expresses the strength-duration measured with the superimposed current pulses. As we have seen already, $B(t)$ is a continuous function of the duration $t$, and varies with $t$ from about 0.2 up to about 0.45; and $r$, the ratio, is an arbitrary constant which can vary in this experimental condition from about $+2.5$ to $-1.0$.

It is of interest that the strength-duration relation $v = F(t)$ cannot be expressed by the same form of function as $f$ (simply by changing its constants), namely, in the form $f(a', b', c', \ldots, t)$. If $V = f(a, b, c, \ldots, t)$ is of hyperbolic form, $v = F(t)$ is not in general a hyperbola when an arbitrary value is chosen for the ratio $r$. Under these experimental conditions, the formula $v = F(t)$ represents the general strength-duration relation; $V = f(a, b, c, \ldots, t)$ is involved in it as being a special case in which $r = 0$. The value $r$ indicates the manner in which the applied potential is distributed along the nerve fiber. *Thus it is evident that the strength-duration curve of a nerve fiber changes simply by changing the distribution of potential along the fiber.*

In the usual experiments with nerve trunks, it never happens that the applied potential drop is localized between two neighboring nodes as in the present experiments with "ridge-insulators." The stimulating current is distributed all along the stretch between and probably around the two electrodes. The strength-duration relation obtained under such circumstances must be much more complicated than those stated above in which the applied potential difference is localized between two or three neighboring nodes. It must involve many constants $r$, $r'$, $r''$, etc., indicating the ratios of the potential differences between nodes of Ranvier, and, consequently, it will have very little meaning, as we know little or nothing about the distribution of potential at the nodes of the nerve fiber in each case.

Many attempts have been made in the past to formulate the law of electric excitation of the nerve, with the hope that all the experimental data may be covered ultimately by a single formula with several constants which characterize the irritability of the tissue. But now it is clear that no simple equation with two or three arbitrary constants can describe the complexity of electric excitation of the "nerve trunk."

III. *Plasma membrane and excitatory process at the nodes of Ranvier.* The original purpose of the present investigation was to examine the electrical properties of the myelin sheath and the significance of the node of Ranvier in the electric excitation of a nerve fiber. It can easily be deduced from the above-cited experimental results that the myelin sheath is a practically perfect electric insulator and consequently that the nodes are the only spots where the excitatory state is built up by an electric current.

In the experiment of figure 2, the observed points in the first quadrant lie on a single straight line AA′ which is approximately symmetrical with respect to both axes, that is,

$$V + V' = \text{constant},$$

where $V$ and $V'$ are the potential differences across the central set and across the distal set of ridge-insulators directed towards the middle pool of Ringer's fluid. It follows directly from this relation that the effects of stimulation with V and V′ (i.e., the excitatory states produced by V and V′) are superposed at a single point on the nerve fiber between the two insulated regions. Since the nerve fiber is apparently continuous except at the node of Ranvier, the node $N_0$ must be the very point where the excitatory states effective as regards excitation are built up by these potential differences. *It is obvious that an electric current excites the nerve fiber only at the node of Ranvier where the applied electromotive force produces an outward-directed potential drop across the plasma membrane.* This conclusion is directly supported by the experimental fact that if, in the arrangement shown in figure 6, A, no node is introduced in the middle pool, the rheobasic voltage can rise up to 1 volt or more.

Since Nernst's theory of nerve excitation, stress has been laid by many investigators upon the semipermeable membrane of the tissue where the ions effective as regards excitation accumulate. Now, there seems no doubt that the short cylindrical surface of the axis-cylinder, not covered by the myelin sheath, is the place where Nernst's polarization occurs. As is clearly shown in the diagram (fig. 9, A), this part of the plasma membrane is the only place where the currents i and i′ flowing along the axis-cylinder can produce a summated effect.

Since the potential difference applied across the ridge-insulator can produce an excitatory state at the node, the myelin sheath cannot be a good electrical conductor. If it were an imperfect insulator, the current flowing along the axis-cylinder would diminish exponentially with increasing distance from the insulated region. Therefore, when the node $N_0$ was situated not exactly in the middle of the pool but, say, nearer to the central set of the ridges (fig. 1, A), the potential difference V′ should be more effective than V. Thus the angle $\alpha$ in figure 2 should vary with the position of the node. But the actual experimental data show that V and V′ are always equally effective and, in consequence, the angle $\alpha$ is approximately equal to 45° (43°–48°). The myelin sheath must therefore be regarded as a *practically perfect insulator.*

We have seen that all the points on the straight line in the first quadrant, including the point A and A′ in figure 2, represent excitation at the node $N_0$. Hence it is evident from symmetry that at the point B and C in figure 2 excitation occurs at the nodes $N_1$ and $N_2$ respectively. From

this it follows directly that the two straight lines passing through the points B and C in the figure represent excitation at these nodes on both sides of $N_0$. Let us consider why the straight line in the second and third quadrant shows, unlike Rushton's experiment on the nerve trunk, a slope far smaller than a right angle.

Since the outward-directed current through the node (or the potential drop across the membrane at the node) is proved to be effective as regards excitation, decrease in the threshold strength for V by superimposition of concurrent V′ (fig. 5) indicates that not only V but also V′ produces an outward-directed current at the node $N_1$. And this will occur when, and only when, the plasma membrane at the node exhibits enormously great electric resistance.

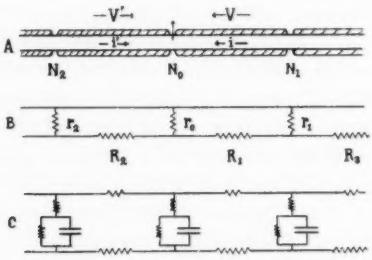



Fig. 9. Diagram representing the electrical circuit in the nerve fiber. *R*'s: resistance along the axis-cylinder between the nodes; it would probably be a homogeneous cylindrical strand of specific resistance of about 80 ohms (Ringer's fluid), about 7 microns in diameter and about 2.5 mm. in length. *r*'s: resistance due to polarization of the plasma membrane at the node. In diagram C, the *r*'s are replaced by leaky condensers and the resistance through the surrounding medium is shown by short zigzags.

Figure 9, B shows the diagram of the electrical circuits in the nerve fiber constituted from these considerations. The symbols $R_1$, $R_2$, $R_3$, ... indicate the resistances of the internodal interval along the core; they would probably be purely ohmic resistances of the order of several tens of megohms. The symbols $r_0$, $r_1$, $r_2$, ... represent the resistances through the plasma membrane at the nodes, due evidently to polarization by the current at this semipermeable membrane. The resistance through the surrounding medium is always very small as compared to R and r, and is omitted in the diagram. If the r's in the figure were negligibly small as compared to those through the axis-cylinder ($R_1$, $R_2$, $R_3$, ...), it would be impossible for the potential difference V′ applied between the node $N_0$ and $N_2$ to produce an excitatory state at the remote node $N_1$. The result shown in figure 5 can be explained only by assuming a great resistance (of the order of ten megohms) through each node of Ranvier.

Since this resistance across the node is considered to be a polarization resistance, its value will augment as the current passes through the membrane. Thus, the longer the duration of the current pulse, the more readily is the excitatory state built up at the remote nodes. This deduction is directly supported by the experimental fact that the angle $\beta$ decreases with increasing pulse duration (fig. 5).

The term "excitatory state" (or excitatory disturbance) here is used to describe the state or condition, without any implication as to its physical nature, which, if it attains a certain constant level, releases an observable nerve impulse. The experimental results that have been described give us some perfectly rigid information as to the manner in which this state is built up at the plasma membrane at the node by the stimulating current.

In the experiment of figure 5, the excitatory state set up at the node $N_1$ by the potential difference V can naturally be considered as a single-valued function of $V$, the voltage, and $t$, the time from the onset of the current pulse. The form of this function is unknown, and we shall denote it by $E(V, t)$. Similarly, the excitatory state built up at the same node $N_1$ by the voltage $V'$ applied between the nodes $N_0$ and $N_2$ may be denoted by $E'(V', t)$. The form of this latter function is of course quite different from that of the former, since $E'(V', t)$ is concerned with the remote effect of the current while $E(V, t)$ describes the process at the node where the current is applied directly.

If the potential differences $V$ and $V'$ are applied concurrently, the excitatory states $E(V, t)$ and $E'(V', t)$ are considered to produce a summated effect. In order that the relation between $V$ and $V'$ for threshold excitation shall lie on a straight line (fig. 5), the following two conditions have to be satisfied.

(1) The resultant excitatory state at the moment $t$ is equal to the sum of $E(V, t)$ and $E'(V', t)$.

(2) The values of $E(V, t)$ and $E'(V', t)$ are directly proportional to the voltage $V$ and $V'$ respectively; that is, if $n$ and $m$ are arbitrary (real) constants, $E(nV, t) = nE(V, t)$ and $E'(mV', t) = mE'(V', t)$.

Thus, when a nerve fiber is just excited with potential differences $V$ and $V'$ of duration $t_0$ applied concurrently, we have

$$E(V, t_0) + E'(V', t_0) = V\,E(1, t_0) + V'E'(1, t_0) = \text{constant},$$

where $E(1, t_0)$ and $E'(1, t_0)$ represent the values of the excitatory states at the moment $t_0$ set up by a unit potential difference and are evidently constant for the given nerve fiber. The last equation describes the linear relation between $V$ and $V'$. The excitatory state can be either positive or negative according to the direction of the potential difference, that is,

according to the sign of $V$ or $V'$. The negative excitatory state follows exactly the same laws as the positive one.

The condition (1) above-cited may be called the *law of superposition*. It states that the excitatory state set up by $V$ and $V'$ applied concurrently is equal to the sum of those which will be produced when $V$ and $V'$ are applied separately. Close analogies are found in physical systems: e.g., the potentials produced by charges on a system of conductors or the current produced by electromotive forces in a net-work of resistances and condensers.

The condition (2) may be called the *law of proportionality*. This, however, cannot be regarded as independent of the former law; it can be involved in the former as being the limiting case in which $V$ and $V'$ are applied at the same region of the nerve fiber. Thus we may state these laws in the following form: When a nerve fiber is stimulated with potential differences $V$, $V'$, ... of duration $t$ applied concurrently between different nodes of Ranvier, the excitatory state (e.s.) produced at a node is a linear function of $V$, $V'$, ...; that is,

$$\text{e.s.} = E\,V + E'V' + \ldots$$

where the coefficients $E$, $E'$, ... are the function of only the pulse duration $t$.

Since we have assumed that the threshold is reached when the excitatory state $E(V, t) = V\,E\,(1, t)$ attains a critical positive value (say $k$), the curve defined by the equation $V = k/E(1, t)$ expresses the strength-duration relation measured with V, which by experiment is closely hyperbolic. Thus the shape of the function $E(V, t)$ can be determined by the observation of the strength-duration relation.

It seems quite natural to consider that the excitatory state reaches the maximum immediately before the break of the applied current. If, however, its maximum is assumed to lag behind the break of the current (Bouckaert and Colle, 1937), it may be necessary to modify the above-stated definition of $E(V, t)$ as the maximum value of the excitatory state produced by a potential difference $V$ of duration $t$, the lag of the maximum being independent of the value of $V$ or $V'$. We shall reserve the problem of the lag for a subsequent paper and shall confine our attention, in this paper, to the previous definition, neglecting such a lag.

Similarly the curve defined by $V' = k/E'(1, t)$ represents the strength-duration relation for the remote effect, i.e., measured with the potential difference $V'$ (fig. 5) when excitation at the node $N_1$ is taken as index. But this cannot be observed directly as excitation occurs at the node $N_0$ at lower voltage than at $N_1$. It can be obtained by extrapolating the straight lines in figure 5 and by determining the points at which these lines cross the vertical axis.

If, in the experimental arrangement shown in figure 5, Ringer's fluid in the middle pool is replaced with a dilute narcotizing solution, it sometimes happens that the broken lines (in fig. 5, left) shift upwards and the continuous lines directly cross the vertical axis, due apparently to rise of threshold at the node $N_0$. Under such conditions, the relation $V' = k/E'(1, t)$ can be determined directly and it gives a chronaxie longer than the normal ("the abnormal prolongation of chronaxie by narcosis").

It is clear from the experiment of figure 5 that $E'(1, t)$ rises more slowly than $E(1, t)$ and approaches, as the time elapses, the maximum, which is 0.45–0.35 times as large as that of $E(1, t)$. The ratio $E'(1, t)/E(1, t)$ gives the slope of the straight line in the second and third quadrants, being equal to the function $B(t)$ introduced to describe the variation in the strength-duration curve with the change of the distribution of potential along the nerve fiber. Dependence of this ratio upon the time $t$ is undoubtedly due to the variable polarization resistance through the plasma membrane.

In the experiment of figure 3, the excitatory state produced at the node $N_0$ by the potential difference $V$ is given by the function $E(V, t)$ and that produced by $V'$ at the same node is also given by the same function $E(V', t)$. Hence, by the law of proportionality and superposition, the relation between $V$ and $V'$ for threshold excitation is

$$VE(1, t_0) + V'E(1, t_0) = k,$$

where $t_0$ is the duration of the current pulse. This equation describes the linear and symmetrical character of the straight lines in the first quadrant.

Discussion. It is evident that the above-stated results obtained with nerve fibers account for the reduction of the chronaxie in the "nerve trunk" by a diminution of the size of the electrode (Davis, 1923; Lapicque, 1931) or by decrease in the distance between electrodes (Rushton, 1927). When the size of the electrode is made small, as in Sakamoto's experiment (1933), the current density becomes very great around the electrode, and the potential difference would be localized roughly between two or three nodes near the electrode. Consequently the strength-duration curve obtained would approach those shown in figure 4. And much the same may be said of the case of very short interpolar distance.

When the size of the electrode applied to the nerve trunk is made greater, potential differences would appear between all the internodal intervals in the interpolar region. But at the region where the gradient of potential is constant, applied current produces no outward-directed potential drop through the node. The positive excitatory states are set up only at the nodes near the cathode. At threshold, a nerve fiber, of which a node happens to be situated at a point most favorable for stimulation, is excited by the sum of the excitatory states set up by potential

differences between the nodes at various distances from the node to be excited. Consequently, the chronaxie thus determined would give a value greater than those obtained in the experiment of figure 8.

Bishop (1928) and others raise the question of deformation of the stimulating current by the polarization of connective tissue which surrounds the nerve. But no attention had hitherto been given to the fact that the connective-tissue sheath produces another, more remarkable, effect upon the nerve fibers inside, namely, the reduction of the gradient of potential near the cathode. Grundfest (1932) found that the strength-duration curve of the individual nerve fiber in the retrolingual membrane varied with the distance of the electrode from the fiber. Kubo and Ono (1934) showed with isolated nerve fibers that the chronaxie measured with the electrode on the myelin sheath (at a point half-way between two nodes) is 2 to 4 times as large as that obtained with the electrode directly on a node. All these facts can be explained by the alteration of distribution of potential along the nerve fiber. The deformation of the stimulating current by the polarization at the connective tissue does not seem to bring about a greater change upon the strength-duration curve than does the reduction of the gradient of potential.

The diagrams of figure 9 showing the electrical circuits in the nerve fiber are of some importance. The figure gives very clear explanations from the physical point of view for some of the well-known phenomena. For example, the *spread of the stimulating current* can be regarded as a logical consequence of this electrical net-work.

When a localized potential difference is applied between two neighboring nodes (e.g., by the use of a ridge-insulator), the positive and negative excitatory state is set up all along the nerve fiber, its magnitude being dependent upon the remoteness from the site of application of the potential difference. At the node near the ridge-insulator, its value is greatest. At the adjoining node, it is, from what has been stated already, cot $\beta$ times as large; and at the $n$-th node it would probably be about $(\cot \beta)^n$ times as large. (The calculation of this value is reserved for a subsequent paper.) If, therefore, the voltage of $1/(\cot \beta)^n$ times the threshold voltage at that duration is adopted for stimulation, the propagated disturbance is expected to start at the $n$-th node from the cathode. This type of current spread can be checked by crushing the nerve fiber and would evidently be what was labeled "internal spreading" by Kato (1926). His "external spreading" probably means the spread of lines of flow in the conductive medium which surrounds the fibers in the nerve trunk.

The electric resistance through the surrounding medium would always be small as compared with the resistances along the axis-cylinder inside the myelin sheath. The action potential we measure seems to be a small fraction of the electromotive force developed at the excitable interface at the node of Ranvier.

## SUMMARY AND CONCLUSIONS

1. By the method of tripolar stimulation, the mechanism of electric excitation of the nerve fiber has been investigated.

2. The plasma membrane at the node of Ranvier (coaxial with the axis) is the place where Nernst's polarization occurs. The resistance through this membrane is inferred to be of the order of ten megohms as measured with the stimulating current.

3. The myelin sheath must be regarded as a practically perfect electric insulator.

4. The excitatory state (or disturbance) is produced by the outward-directed potential-drop across the plasma membrane at the node. The "law of proportionality and superposition" (p. 392) applies to this state.

5. When a potential difference is applied to a nerve fiber between two neighboring nodes of Ranvier, excitatory states are produced all along the fiber. Spread of the stimulating current in the nerve trunk is explained upon a physical structural basis.

6. The strength-duration curve varies with the distribution of potential along the nerve fiber. Variability of the resistance through the plasma membrane accounts for this fact. The dependence of the chronaxie upon the size of the electrode and its allied phenomena are explained from this standpoint.

It is my wish to express to Prof. G. Kato and Dr. R. Kawakami my appreciation of their continual encouragement and valuable advice. To Dr. H. Davis my thanks are due for his kindness in improving the English.

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# THE GONADOTROPIC CONTENT OF THE HYPOPHYSIS THROUGHOUT THE LIFE CYCLE OF THE NORMAL FEMALE RAT[1]

H. D. LAUSON, JUNE B. GOLDEN AND E. L. SEVRINGHAUS

*From the Department of Medicine, University of Wisconsin Medical School, Madison*

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With the exception of the paper by Clark (1), there has been no report of a comprehensive study of the hypophyseal gonadotropic content through the life cycle of the normal female. Most of the studies, made on several species and using various methods of assay, have been confined to one or more isolated periods in the life history, such as fetal, infantile, puberal, adult and senile states (2, 3, 4, 5, 6, 7). The present investigation was undertaken to study systematically the periods of infancy, pre-puberty, puberty, adulthood and senility, using a single strain of rats and constant technics.

METHODS. Normal healthy female rats of the Sprague-Dawley strain[2] were used throughout. They were sacrificed at ages ranging from 14 days to about 2½ years. The whole pituitary glands of each age group were pooled immediately after removal, and were macerated and suspended in water, as described by Heller, Lauson and Sevringhaus (8). These suspensions were assayed immediately for gonadotropic content by the uterine method. Twenty-two day old female rats, 34 to 40 grams in weight, were employed as test animals. Subcutaneous injections of 0.5 cc. each, were made twice daily for three days, with autopsy on the morning of the fourth day (74 to 76 hours after the first injection). Between injections the suspensions were stored at 4°C. Weights of the ovaries and uteri (after fluid had been expressed) and vaginal opening were recorded at autopsy. The mean uterine weight of each group of test animals was referred to a standard dosage-response curve, and the number of uterine units per dose was read directly from the abscissa. Knowing the average pituitary weight of the donor rats, and the size of the dose in mgm. of pituitary tissue, the number of uterine units of gonadotropic activity per average hypophysis was readily calculated for each age group.

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[2] We are indebted to Sprague-Dawley, Inc. for gratuitously furnishing the adult rats used in this study.

The standard dosage-response curve was constructed from results of the assay of pooled pituitaries from 25-day old normal females. Four groups of 12 test rats each were injected with total doses of $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$, and 1 gland-equivalent, respectively. The mean uterine and ovarian weights are shown in figure 1. The smooth curve drawn through the means has been taken as the curve of reference. The vertical bars through the means represent the standard errors of the means, and give a measure of the accuracy of the curve. The abscissa scale was so arranged that 1 unit represented a uterine weight of 53 mgm. This unit is purely arbitrary and has no significance beyond the scope of this paper. Although this curve is based on immature female hypophyseal material, we believe that the slope and form would probably not differ significantly if pituitaries from normal adult females had been used as the standard.

To avoid any possible variation in hypophyseal potency due to the estrous cycle, all the mature rats, except the senile group, were sacrificed in the proestrous stage. The rats were smeared only on the day of autopsy, and only those showing the typical smear of nucleated cells were selected. Later, at autopsy, any rat the uterus of which was not distended by fluid was discarded. The proestrous stage was chosen because it is the shortest and most sharply defined phase of the cycle. By this selection, we believe that we have placed all adult groups on a comparable basis.

Preliminary experiments were conducted to guide in the selection of doses which could be expected to fall into the useful range of the dosage-response curve.

Results. Table 1 summarizes the results of this study. The left-hand columns present the essential data concerning the donor females. The age at autopsy, number of rats sacrificed, and the mean pituitary weights are given. All rats 35 days of age or less were immature (closed vaginas, infantile ovaries and uteri), while all those 87 days of age and older were killed in proestrus. The five groups ranging from 49 to 52 days of age represent the puberal period and will be referred to later. The last group of three rats represents the senile age. These were known to be at least $2\frac{1}{2}$ years old, but the exact age was unknown.

The remaining columns of table 1 are devoted to the test animals. The *total* dose administered in the six injections is given in terms of average gland-equivalents as well as in milligrams of whole fresh gland. It will be noted that in case of the 14, 21, and 35 day old groups two dose levels were used in estimating the potency per gland. As mentioned above, the 25-day old group served as the standard to which all other results were referred. The final column shows the number of uterine units contained in 10 mgm. of whole pituitary, these being estimates of the potency of equivalent weights of tissue.

Figure 2 shows the results of the assays expressed as uterine units per

average gland. It is seen that the immature hypophyses are considerably more potent than all adult groups with the exception of the senile group. When expressed as potency per 10 mgm. of tissue, as in table 1, the results are even more striking.

TABLE 1

*Summary of results of assays of pituitaries from normal female rats throughout the life cycle*

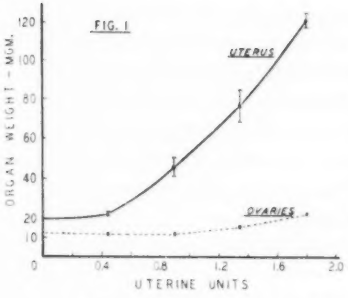
| DONORS | | | | TEST ANIMALS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Period | Age of donors | No. of donors | Mean donor pit. weight | No. of test rats | Total dose of pituitary | | Uterine weight | | Ovary weight | | Uter. units per dose | Uter. units per aver. gland | Uter. units per 10 mgm. gland |
| | | | | | Gland equiv. | Mgm. | Mean | S.D. | Mean | S.D. | | | |
| | *days* | | *mgm.* | | | | *mgm.* | | *mgm.* | | | | |
| Sexual immaturity | | | | 55 | 0—Controls | | 19.6 | ±2.6 | 12.6 | ±1.8 | | | |
| | 14 | 21 | 1.7 | 10 | 0.5 | 0.85 | 21.5 | ±2.3 | 11.6 | ±1.6 | 0.4 | 1.0 | 6.0 |
| | | | | 10 | 1.0 | 1.7 | 52.9 | ±18.3 | 13.6 | ±2.1 | 1.0 | | |
| | 21 | 21 | 2.0 | 12 | 0.5 | 1.0 | 71.4 | ±26.2 | 16.6 | ±4.0 | 1.3 | 2.6 | 13.0 |
| | | | | 12 | 1.0 | 2.0 | 113.3 | ±9.8 | 33.8 | ±11.8 | >1.8 | | |
| | 25 | 37 | 2.6 | 12 | 0.25 | 0.65 | 22.0 | ±2.4 | 11.3 | ±1.3 | 0.45 | 1.8 | 7.0 |
| | | | | 12 | 0.5 | 1.3 | 45.9 | ±16.5 | 12.0 | ±1.2 | 0.9 | | |
| | | | | 12 | 0.75 | 1.95 | 76.7 | ±28.8 | 15.6 | ±3.2 | 1.35 | | |
| | | | | 12 | 1.0 | 2.6 | 121.9 | ±13.7 | 22.3 | ±4.6 | 1.8 | | |
| | 35 | 20 | 3.2 | 12 | 0.5 | 1.6 | 27.3 | ±6.1 | 13.0 | ±2.7 | 0.6 | 1.4 | 4.3 |
| | | | | 12 | 1.0 | 3.2 | 99.6 | ±26.2 | 17.9 | ±5.2 | 1.6 | | |
| Puberty | 49* | 9 | 5.2 | 8 | 1.0 | 5.2 | 63.1 | ±15.2 | 12.9 | ±1.5 | 1.2 | 1.2 | 2.3 |
| | 50* | 14 | 4.5 | 12 | 1.0 | 4.5 | 62.1 | ±12.4 | 12.7 | ±2.3 | 1.2 | 1.2 | 2.4 |
| | 50* | 6 | 5.5 | 5 | 1.0 | 5.5 | 55.8 | ±17.6 | 12.7 | ±0.8 | 1.1 | 1.1 | 2.0 |
| | 52† | 16 | 6.5 | 12 | 1.0 | 6.5 | 27.5 | ±9.5 | 11.2 | ±1.7 | 0.6 | 0.6 | 0.9 |
| | 49† | 11 | 6.9 | 11 | 1.0 | 6.9 | 20.5 | ±2.1 | 12.4 | ±2.2 | <0.4 | <0.4 | <0.6 |
| Sexual maturity | 87 ±1 | 25 | 7.4 | 12 | 2.0 | 14.8 | 67.9 | ±15.7 | 14.4 | ±2.6 | 1.2 | 0.6 | 0.8 |
| | 113 ±2 | 25 | 10.7 | 12 | 2.0 | 21.3 | 76.1 | ±15 4 | 13.7 | ±2.0 | 1.3 | 0.7 | 0.7 |
| | 169 ±3 | 20 | 13.2 | 12 | 1.5 | 19.8 | 45.1 | ±11.4 | 13.0 | ±1.3 | 0.9 | 0.6 | 0.5 |
| | 276 ±5 | 20 | 16.1 | 12 | 1.5 | 24.1 | 54.5 | ±18.4 | 13.5 | ±2.2 | 1.0 | 0.7 | 0.4 |
| Senility | >2½ yr. | 3 | 16.6 | 5 | 0.5 | 8.3 | 102.7 | ±27.2 | 14.0 | ±5.3 | 1.6 | 3.2 | 1.9 |

S.D. = Standard Deviation = $\sqrt{\frac{S(x-\bar{x})^2}{N-1}}$

* Pre-puberal rats with closed vaginas and immature ovaries and uteri.

† Puberal rats with open vaginas and developed ovaries and uteri. (See table 2.)

Special attention was devoted to the study of the gonadotropic content of the hypophysis in relation to puberty. Five groups of rats, 49 to 52 days of age, were sacrificed on three different days. They were grouped on the basis of sexual development, as shown in table 2. There were three groups in which vaginal introitus had not been established. The ovary weights were less than 20 mgm. in most of the animals of the first two groups, and the uteri weighed less than 100 mgm. The third group



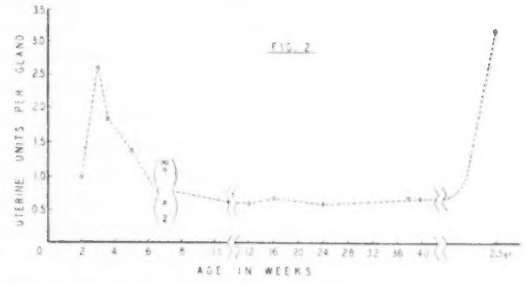




Fig 1. Standard curve of reference for gonadotropic assays, based on uterine and ovarian weights in test rats injected with $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and 1 gland-equivalents of pituitary from 25-day-old normal female rats. The abscissa scale is graduated in terms of an arbitrary uterine unit. The vertical bars represent the standard error of the mean.

Fig. 2. Hypophyseal gonadotropic content of normal female rats, expressed in terms of uterine units per gland. The abscissa represents age of donors. The brackets enclose the three pre-puberal (upper) and the two puberal assays (lower). The curve is deliberately drawn indefinitely through these points, since the gonadotropic content of the pituitary at this age depends inversely on the degree of sexual development. (See table 2 for details.)

TABLE 2

*Details of the puberal and pre-puberal groups*

| | DONORS | | | | | | | | TEST RATS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Uterine weight | | Ovary weight | | | | | Uterine weight | | Ovary weight | | |
| | Group | No. of rats | | Mean | Min.-Max. | Mean | Min.-Max. | Vaginas open | No. of rats | Total dose | Mean | Min.-Max. | Mean | Min.-Max. | Uter. units per gland |
| | | | *days* | | | | | | | | | | | | |
| Pre-puberal | 1 | 9 | 49 | 57.1 | 38.8<br>92.6 | 17.1 | 14.1<br>21.3 | None | 8 | 1 gl. | 63.1 | 35.3<br>80.1 | 12.9 | 10.7<br>14.7 | 1.2 |
| | 2 | 14 | 50 | 63.7 | 41.8<br>91.8 | 15.8 | 13.0<br>23.2 | None | 12 | 1 gl. | 62.1 | 42.0<br>86.0 | 12.7 | 8.9<br>14.2 | 1.2 |
| | 3 | 6 | 50 | 169.5 | 120.2<br>201.6 | 20.4 | 19.3<br>22.4 | None | 5 | 1 gl. | 55.8 | 38.7<br>85.7 | 12.7 | 12.0<br>13.9 | 1.1 |
| Puberal | 4 | 16 | 52 | 153.4 | 96.6<br>264.6 | 30.6 | 19.2<br>40.0 | All* | 12 | 1 gl. | 27.5 | 21.7<br>56.0‡ | 11.2 | 8.9<br>14.2 | 0.6 |
| | 5 | 12 | 49 | 164.0 | 110.0<br>302.2 | 35.0 | 28.8<br>44.4 | All† but one | 11 | 1 gl. | 20.5 | 17.2<br>25.0 | 12.4 | 9.3<br>17.3 | <0.4 |
| | | | | | | | | | 55 | Control | 19.6 | 15.1<br>26.8 | 12.6 | 8.9<br>16.8 | |

* Of the 16 rats, 11 vaginas opened within five days before autopsy, and the remaining five opened earlier.

† Of the 11 rats, 7 vaginas opened within five days preceding autopsy, and the other four opened earlier. The rat whose vagina had not opened, had 28 mgm. ovaries, and was included in the puberal group for that reason.

‡ Eleven of these 12 uteri weighed less than 33 mgm.

of pre-puberal rats included all rats having closed vaginas, but in which the uterine weight exceeded 100 mgm. These rats were considered to be on the threshold of puberty. The last two groups consisted of animals in which ovaries, uteri and vaginas were definitely puberal. That is, vaginas were open at autopsy, uteri weighed more than 96 mgm., and the ovarian weights were well above the pre-puberal level. It is interesting to note that in several instances the heavier uteri (fluid removed) were associated with the lighter ovaries. For example, in group 4, the rat with the lowest uterine weight, 96.6 mgm., had 36.2 mgm. ovaries, while the one with 19.2 mgm. ovaries had a 188 mgm. uterus.

The right-hand columns of table 2 give the results of the gonadotropic assays. In all cases a total of one gland-equivalent was injected into the test rats. Although the uterine weights of the test animals of any one group showed considerable variation, the difference between the three pre-puberal groups, on one hand, and the two puberal groups, on the other, is perfectly clear. The uterine responses in group 5 did not differ from the untreated controls, so the potency per gland is given as less than 0.4 unit. It is seen that the average gland content in the pre-puberal groups is at least twice that of the puberal groups of the same age. Comparison of the mean uterine weights of the test animals (index of the gonadotropic content of the donor hypophysis) with the average ovarian weights of the donors (index of the degree of sexual development in the donor) reveals a definite inverse correlation.

The three senile rats were the survivors of a group of old virgin females maintained in the colony for several years. About a year before autopsy, this group had been smeared daily for several three-week periods. At this time many already appeared to be non-cyclic. Unfortunately, the three were not smeared prior to autopsy, hence it is impossible to state with certainty that they were beyond the climacteric. At autopsy, two of the animals were found to have atrophic uteri and fibrotic-appearing ovaries without visible recent corpora lutea. The uterus of the third rat was partly atrophied, but the ovaries contained recent corpora. Hence, we may say that the rats were very old, and that perhaps two were past the climacteric. That the pituitaries of these rats were three to four times as potent as the pituitaries of the adult animals in proestrus is quite definite. Individual assays done on three senile female rat hypophyses a year ago yielded results of about 1.4, 1.1, and 2.3 uterine units per gland. One of these rats was in proestrus, and one had been acyclic for three weeks prior to autopsy. Hence, it seems reasonably established that the female hypophysis becomes more potent in old age. More adequate study along this line is planned for the future.

Since referring results of assays to a standard dosage-response curve of the type employed in this study introduces additional error, it is essential

to verify the above conclusions by direct critical examination of the uterine and ovarian weights of the test animals. Table 3 presents the mean weights of the uteri and ovaries, with their standard errors, arranged with respect to dose in gland-equivalents. The differences between the 14 and and 21 day groups, between the 21 and 25 day groups, and between the 21, 25 and 35 day groups, on one hand, and the pre- and post-puberal groups, on the other, are clearly significant. It appears safe to conclude that the gonadotropic content of the immature and pre-puberal hypophyses is

TABLE 3

*Mean uterine and ovary responses of test animals receiving comparable doses in terms of gland-equivalents*

| AGE OF DONORS | DOSE IN GLAND-EQUIVALENTS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ½ gland | | 1 gland | | 1½ glands | | 2 glands | |
| | Uter. wt. | Ovary wt. | Uter. wt. | Ovary wt. | Uter. wt. | Ovary wt. | Uter. wt. | Ovary wt. |
| *days* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| 14 | 21.5 ±0.7 | 11.6 ±0.5 | 52.9 ±5.8 | 13.6 ±0.7 | | | | |
| 21 | 71.4 ±7.7 | 16.6 ±1.2 | 113.3 ±2.9 | 33.8 ±3.5 | | | | |
| 25 | 45.9 ±4.8 | 12.0 ±0.4 | 121.9 ±4.0 | 22.3 ±1.3 | | | | |
| 35 | 27.3 ±1.8 | 13.0 ±0.8 | 99.6 ±7.7 | 17.9 ±1.5 | | | | |
| 49* | | | 63.1 ±5.4 | 12.9 ±0.5 | | | | |
| 50* | | | 62.1 ±3.6 | 12.7 ±0.7 | | | | |
| 50* | | | 55.8 ±7.9 | 12.7 ±0.4 | | | | |
| 52† | | | 27.5 ±2.7 | 11.2 ±0.5 | | | | |
| 49† | | | 20.5 ±0.6 | 12.4 ±0.6 | | | | |
| 87 | | | | | | | 67.9 ±4.5 | 14.4 ±0.8 |
| 113 | | | | | | | 76.1 ±4.4 | 13.7 ±0.6 |
| 169 | | | | | 45.1 ±3.2 | 13.0 ±0.4 | | |
| 276 | | | | | 54.5 ±5.3 | 13.5 ±0.6 | | |
| 2½ yr. | 102.7 ±12.2 | 14.0 ±2.4 | | | | | | |

* Pre-puberal.

† Puberal. These data are taken from table 1, and are arranged to permit direct comparison of uterine and ovarian responses to similar doses. It should be emphasized that the doses are in terms of gland-equivalents of the hypophyses from the respective donor age groups, and that they do not, therefore, represent equivalent weights of pituitary tissue.

The standard error of the mean was calculated from: $\text{S.E.} = \frac{\text{S.D.}}{\sqrt{N}}$

significantly greater than in the puberal and adult groups, excepting the senile.

Discussion. It should be pointed out that the uterine method of assay probably does not measure only the FSH activity of a gonadotropic preparation, as might be inferred from the paper by Levin and Tyndale (9). Studies in this laboratory indicate that when hypophyseal LH is injected together with pituitary FSH a greater uterine weight is produced than with the same amount of FSH alone. The uterine weight method of gonadotropic assay thus appears to be no more specific for FSH than the ovarian weight method. The greater sensitivity of the former is still a real advantage.

The assays described above, therefore, do not yield any *qualitative* information about the gonadotropic complex of the pituitary. Furthermore, they are quantitative only insofar as they measure the indirect effect of the total complex upon the immature uterus. No information concerning the influence of age and sexual maturity upon the absolute and relative amounts of FSH, LH, and other postulated factors of the anterior lobe is available at present.

It is important to emphasize the fact that in these assays, all hypophyses from a given age group were pooled together and made into one homogeneous suspension. All variation in the donor glands was thus arbitrarily eliminated, leaving as the only important variable the response of the test animals. We feel that the order of donor variation within any of the age groups would probably be less than the differences between them. Since the variation in uterine weight of the test animals is quite large (see S. D. in table 1), the use of fairly large numbers of recipients is necessary to insure reasonable accuracy in estimating the units per average gland. Except in a few groups, at least 10 test animals have been employed on each dose. We believe, therefore, that the error is relatively small.

It should also be noted that in spite of the relatively large numbers of rats in each group, the assays represent only single determinations. The prepuberal and puberal groups alone have been repeated. The consistency found in these five groups, however, is significant.

For a discussion of the earlier work indicating the high gonadotropic content of the immature female hypophysis, the reader is referred to the paper by Clark (1). Many of these results, including Clark's, were obtained by implantation of pituitary tissue into test animals. For reasons discussed in a previous communication (8), we believe that the suspension method here employed represents a genuine refinement of technic.

These results confirm Clark's observation that a peak of hypophyseal potency occurs during the third week of life. We can add that the content per gland at 21 days is actually about twice as great as at 14 days. Following this peak, there is a steady decline till at seven weeks the hormone content of the prepuberal gland has dropped to about the level of the 14 day hypophysis. Then with the appearance of puberty, the potency drops, perhaps quite rapidly, to about half of the pre-puberal level.

This abrupt drop in potency at puberty may be explained by either of two hypotheses: *a*, the rapid increase in estrogen secretion at puberty inhibits the formation of hypophyseal gonadotropic complex; or *b*, this same increased estrogen secretion causes more rapid release of previously stored hormone from the anterior lobe. We incline to the latter view, largely because of the work of Fevold, Hisaw, and Greep (10) and Hohlweg (11) who showed that estrin releases LH from the hypophysis. The observations of Fevold, Hisaw and Greep (12), that injection of gonadotropic

preparations into normal test rats produced a greater ovary weight increase than in hypophysectomized test animals, may be explained on the assumption that the injected hormone causes the ovaries to secrete estrin, which, in turn, releases additional hormone (LH ?) from the rat's own pituitary, augmenting the action of the injected material. Recently Tyndale, Levin and Smith (13) have shown that the immature animal's pituitary participates in the ovarian response to injected menopause urine extract.

In their report of parabiotic studies, Meyer and Hertz (14) state that "It is . . . difficult to visualize the normal approach of puberty except in terms of increasing gonadotropic potency of the pituitary gland without proportionally greater estrone secretion by the pre-pubertal ovary." Our data would show that the potency decreases considerably as puberty approaches. This may mean, however, that the quantity released into the blood stream actually increases during this period of development. Long and Evans (15) showed that immature ovaries transplanted to an adult female rapidly grew to adult size and assumed cyclic activity, indicating that the adult hypophysis actively secretes gonad-stimulating hormone into the blood stream, in spite of the fact that the hormone complex contained in the gland at any time is at a low level. Our results indicate that the amount in the gland during proestrus from the 12th to the 40th week of life is remarkably constant.

Our observations on senile rats confirm those of Smith and Engle (7). The hypophysis in this period becomes more potent as the ovaries fail. The parallel between natural and artificial climacteric is strongly suggested by these results, although studies in this laboratory (to be published) show that the increase in pituitary potency following ovariectomy is much more rapid. Concerning the physiology of the hypophyseal-ovarian relationship in the climacteric, there are apparently conflicting reports indicating hypersecretion of gonadotropic hormone, on one hand (14, 16, 17, 18), and a capacity of senile ovaries to be reactivated by gonad-stimulating hormones, on the other (19, 20). More research is needed to harmonize these findings.

We are grateful to Doctors R. K. Meyer and Roy Hertz of the Zoology Department for their helpful suggestions and criticisms.

## SUMMARY

The gonadotropic content of the normal female rat hypophysis in infancy, pre-puberty, puberty, adult sexual life and senility has been investigated. Pituitaries from 248 donors, ranging from 14 days to 2½ years of age, were assayed by the uterine method on 272 immature test rats, 22 days old. All adult rats were sacrificed in proestrus. The pitui-

tary potency rises to a peak at 21 days and decreases gradually until the onset of puberty. At puberty, there is a rapid drop in potency to about one-half of the pre-puberal level. Throughout adult sexual life, the content of the hypophysis in the proestrous stage remains constantly at the low puberal level, despite continued increase in pituitary size. This is interpreted to mean that the adult gland stores very little of its gonad-stimulating complex, but releases it as rapidly as formed. Limited study of the senile age has revealed that the potency markedly increases as the ovaries fail. The assumed parallel between the natural and artificial climacteric seems reasonably established.

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# IS THE PHYSIOLOGICAL ACTIVITY OF POTASSIUM DUE TO ITS NATURAL RADIOACTIVITY?

ANTHONY J. GLAZKO AND DAVID M. GREENBERG

*From the Division of Biochemistry, University of California Medical School, Berkeley*

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The radioactivity of purified potassium salts was discovered over 30 years ago by Campbell and Wood (3). At first, it was surmised that all the potassium isotopes were active, but it is now possible to ascribe all the activity to the isotope $K^{40}$ (15, 17), which constitutes only about 0.01 per cent of the naturally occurring potassium (8). Brewer (2) has recently recalculated the half life period of $K^{40}$ and obtained the value $1.67 \times 10^8$ years. The disintegration of $K^{40}$ yields the element calcium plus beta particles and gamma radiation. The origin of Ca from $K^{40}$ seems of interest in connection with the physiological antagonism of these elements. However, it should be noted that the amount of calcium formed in any ordinary interval of time is extremely minute. The reaction involved is:

$$_{19}K^{40} \longrightarrow {}_{20}Ca^{40} + {}_{-1}\beta^{\circ}$$

In spite of the very long half life, the emitted rays possess considerable energy. Two types of $\beta$ particles are emitted, with potentials of 0.3 and 0.7 M.e.v.,[1] respectively, and in the proportion of 60 to 28 (1, 7). Potassium also gives off a very hard $\gamma$ radiation to the extent of about 1 per cent of the total (9).

It has been proposed by Zwaardemaker (18) that this natural radiation is the source of the unique physiological properties of potassium, such as the so-called antagonism between potassium and calcium ions. As an apt example may be cited the common physiological experiment in which an isolated frog heart may be kept beating for hours so long as it is perfused with a saline solution containing potassium and calcium ions in the proper ratio. When there is insufficient potassium present, the heart will stop in a dilated state. Addition of the proper amount of potassium to such a heart will cause it to start beating again.

In support of his hypothesis that the physiological action of potassium is due to its radiation, Zwaardemaker carried out experiments which appeared to show that any source of $\alpha$ or $\beta$ radiation was capable of replacing

[1] Million electron volts.

potassium in balancing the effects of calcium. Thus he reported that the frog heart could be kept beating if the potassium ions were replaced with salts of the heavy radioactive elements such as radium, uranium, and thorium if used in proper concentrations as determined by experimental trial. The amounts of these radioactive metals required to replace potassium were roughly proportional to their radioactivity. From the work of Zwaardemaker it appeared that sufficient radiation to supply a definite kinetic energy value was necessary to maintain the frog heart beating.

The view that the physiological activity of potassium is due to its radioactivity has met with opposition from Clark (4), Libbrecht (11), and Loeb (12), but, on the other hand, it has received favorable support from the work of Gunzburg (5), Hamburger and de Waard (6), and Loew (13).

To test the hypothesis of Zwaardemaker in a new and more rigid fashion, the authors carried out experiments in which potassium was replaced by the artificially radioactive sodium of mass 24. These experiments consisted of perfusing the frog heart with iso-osmotic saline solutions. The frogs employed were kept at 5° for about a week before being used. The hearts were cannulated through the sinus and secured by ligation.

The control perfusion solution was prepared to have the following composition:

| | *per cent* |
|---|---|
| NaCl | 0.65 |
| $NaHCO_3$ | 0.02 |
| $NaH_2PO_4$ | 0.001 |
| $CaCl_2$ | 0.012 |
| KCl | 0.014 |
| pH | 7.4 |

The perfusion pressure was maintained at 8 cm. of water.

The perfusion fluids were oxygenated by vigorous shaking before being used. With the control solution, rhythmic beating went on for hours. However, when the perfusion was carried out with Ringer's solution from which potassium had been omitted, the hearts stopped in diastole after a period of about 30 minutes. On replacing the potassium with varying concentrations of $Na^{24}Cl$, the hearts sometimes gave a few isolated contractions, but never resumed the normal beat. The quantities of $Na^{24}Cl$ added to the perfusion fluids did not appreciably change the total electrolyte concentration. If now the perfusion was taken up with the control Ringer's solution containing KCl, normal beating commenced within a few minutes and could be kept up for hours.

If normally beating hearts were perfused with potassium deficient perfusion solutions which contained varying concentrations of $Na^{24}Cl$, the hearts would gradually stop in diastole just the same as with solutions

Fig. 1

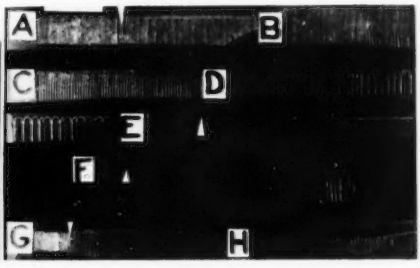


Fig. 2

Fig. 1. Perfusion experiment showing that the heart beat cannot be restarted with radio-sodium.

Explanation: Length of bar at the top represents 1 minute time interval. Top of the stroke is diastole. Changes in perfusion fluids are indicated by the markers on the curves.

Section A: Normal heart beat to first marker on perfusion with balanced Ringer's solution. Perfusion with potassium deficient solution started at marker to stop the heart.

Section B: Heart is stopped by lack of potassium after 30 minutes. At the marker perfusion is started with solution containing $Na^{24}Cl$ sufficient to yield radiation energy of $10^{-11}$ ergs per second per ml. A few isolated beats were obtained.

Section C: Heart perfused with fluid containing $Na^{24}Cl$ sufficient to yield radiation energy of $10^{-9}$ ergs per second per ml. The higher concentration of $Na^{24}Cl$ induced a few more isolated beats. The concentrations of $Na^{24}Cl$ in these fluids were too minute to have an appreciable influence on the total electrolyte concentration.

Section D: Heart perfused with control solution containing potassium. The heart beat is restarted.

Section E: Final condition of the heart showing normal heart beat, $1\frac{1}{2}$ hours after the start of the experiment.

Fig. 2. Perfusion experiment showing that the heart beat cannot be maintained by radio-sodium in the absence of potassium.

Explanation: Length of bar at the top represents 1 minute time interval. Top of the stroke is diastole. Changes in perfusion fluids are indicated by the markers on the curves.

Section A: Normal heart beat on perfusion with balanced Ringer's solution. Perfusion with potassium deficient solution started at marker.

Section B: 5 minutes after starting perfusion with potassium deficient solution.

Section C: 15 minutes after.

Section D: Heart is stopped 20 minutes after start of perfusion.

Section E: Stopped heart perfused with fluid containing $Na^{24}Cl$ sufficient to yield radiation energy of $10^{-8}$ ergs per second per ml. No heart beats obtained.

Section F: Potassium chloride now added to the perfusion fluid. The heart beat is restarted.

Section G: Heart perfused with potassium deficient solution containing $Na^{24}Cl$ sufficient to yield radiation energy of $10^{-8}$ ergs per second per ml.

Section H: The heart beat is again stopped 9 minutes later.

deficient in potassium but containing calcium ions. *Under the conditions of the present experiments, the radioactivity of sodium could not replace the normal physiological action of potassium in maintaining the beating of the frog heart.*

Kymograph records illustrative of these experiments are given in figures 1 and 2.

The same results as with radio-sodium were obtained using radioactive phosphorus (as $NaH_2PO_4$) and radio-potassium ($K^{42}$) in place of natural potassium. In the experiments with radio-potassium, the concentration was kept to less than 1 per cent of the potassium content of the control perfusion fluid, while the intensity of the radiation was varied to between 1000 fold less to 100 fold greater than that of natural potassium.

Using the data then available (18), Zwaardemaker calculated that the energy of radiation from a gram of potassium is about $3 \times 10^{-2}$ ergs per second. This value is undoubtedly too high. With the aid of a Geiger counter, Mühlhoff (14) has determined that the average number of disintegrations from a gram of potassium is about 23 per second. Taking the mean energy of the $\beta$ radiation as 0.5 M.e.v., it may be calculated that the energy of radiation from a gram of potassium is about $1.8 \times 10^{-5}$ ergs per second. Since the potassium content of human blood plasma is about 0.015 per cent, the radiation energy per ml. of plasma should be approximately $2.7 \times 10^{-9}$ ergs per second. A milliliter of whole blood would give about $3 \times 10^{-8}$ ergs per second, or about 0.05 disintegration per second per ml. of blood. The radiation value accepted by Zwaardemaker would give about 74 disintegrations per second per ml. of blood. The radiation energy of the concentrations of $Na^{24}$ used in the present experiments ranged from $10^{-12}$ to $10^{-6}$ ergs per second per ml. of perfusion fluid.

Radio-sodium gives off a $\beta$-particle in its disintegration with an energy of 2 to 3 times that of $K^{40}$ (1.2 M.e.v.) (10), besides a $\gamma$-radiation of considerable intensity (16). The $\gamma$ radiation is not an interfering factor in the action of $Na^{24}$, since $K^{40}$ also gives off $\gamma$ radiation of comparable intensity (9).

In his experiments, Zwaardemaker found that $\gamma$ radiation had no effect on the beating of the frog heart. Even if some physiological effect were postulated due to the possible production of $\beta$-rays by nuclear bombardment, the order of magnitude of this would be about the same in both $Na^{24}$ and $K^{40}$.

Zwaardemaker reported that $\alpha$ and $\beta$ radiation were equally effective in replacing the physiological action of potassium on the frog heart, but that they antagonized each other. Thus if $\alpha$ and $\beta$ radiation were properly adjusted, they would counterbalance each other and would not maintain

the beating of the heart. This would appear to indicate that the action of the radiation was dependent upon the electrical charge.

From the experiments reported here, it appears unlikely that the normal physiological action of potassium is due to its radioactivity. More decisive evidence will have to be offered before the Zwaardemaker hypothesis can be accepted.

We gratefully acknowledge our indebtedness to Professor E. O. Lawrence and the staff of the Radiation Laboratory of the University of California for supplying us with the radio-sodium, phosphorus, and potassium used in these experiments. This was made available by a grant from the Research Corporation and through assistance of the W. P. A.

## SUMMARY

It has been found that neither radio-sodium, minute amounts of radio-potassium, nor radio-phosphorus can replace natural potassium in maintaining the beating of the isolated frog heart. This observation throws in doubt the hypothesis of Zwaardemaker that the radioactivity of natural potassium is responsible for its unique physiological properties.

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# THE PHYSIOLOGICAL ACTION OF NATURAL AND ARTIFICIAL RADIOACTIVITY[1]

JOSEPH G. HAMILTON AND GORDON A. ALLES

*From the Radiation Laboratory, University of California, Berkeley; and the Divisions of Medicine and Pharmacology, University of California Medical School, San Francisco*

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The recent use of many artificial radioactive isotopes of the biologically important elements for therapeutic and metabolic studies raises the question of possible physiological and pathological changes that these substances might produce. Scott and Cook (1) and Lawrence (2) have observed the morphological changes in animals and man that take place following the administration to them of these artificially prepared substances in relatively large quantities. They have found that the most radio-sensitive tissues, such as the hematopoietic system, lymphoid tissue, and the intestinal epithelium, are also the first to be affected following the administration of relatively large doses of these artificial radioactive isotopes. No immediate toxic reactions were observed in either the experimental animals or the human patients following the use of these radio-elements.[2]

It was felt that it would be of interest to compare the physiological action of several of the elements that can exist in the body in the highly ionized state with the effect of equivalent dosages of their corresponding artificial radioactive isotopes. For this purpose sodium, potassium, and rubidium were selected. To serve as a basis of comparison, should any effect be noted, a solution of radium emanation was also used. Particular interest was centered upon comparison of the physiological action of natural potassium and rubidium with that of their artificial radioactive isotopes because of the claims of Zwaardemaker (3) and supported by Gunzburg (4), Hamburger and de Waard (5), and Loew (6), that the physiological properties of these two elements were due to their natural radioactivity. Zwaardemaker stated that the potassium of Ringer's solution could be replaced by equiradioactive quantities of any of the other

[1] This work has been supported by the Josiah Macy Jr. Foundation and the Christine Breon Fund.

[2] The prefix "radio"- indicates that the element has been made artificially radioactive.

radioactive elements when the solution was used for the perfusion of the excised frog's heart. He also observed that an excess of the radioactive material in the perfusing fluid provoked toxic manifestations similar to those produced by a high concentration of potassium. From these observations he concluded that the action of potassium in aiding the automatic contractions of the heart was due to the radioactivity of that element.

METHOD OF STUDY. Dogs were used as the experimental animals in the following studies. Both the natural and activated elements were administered in solutions of their chlorides intravenously and intra-arterially. The radium emanation was used in a solution of isotonic sodium chloride. Continuous kymographic recordings of respiration, pulse, and blood pressure were taken throughout each experiment. The pulse and blood pressure values were obtained by the use of a carotid cannula. In addition to the types of recording listed above, electrocardiographic tracings were made during the first two experiments.

The artificial radio-elements used in these experiments were prepared by deuteron bombardment in the Berkeley cyclotron. The radio-potassium was made by the deuteron bombardment of potassium chloride except that used in the last experiment, for which the potassium was activated as the metal and then converted to the chloride. Rubidium chloride was bombarded to produce the radio-rubidium, and the radio-sodium was prepared from the metal which was then converted to the chloride. The radioactivity of the three radio-elements and of the radium emanation was measured with a Lauritsen type quartz fiber electroscope which had been previously calibrated against a radium standard.

The radio-potassium was administered in the first experiment intravenously and intra-arterially in the form of a 3 per cent solution of potassium chloride since its activity was quite weak. The sample of radio-potassium for the second experiment was stronger, and in this instance was administered intravenously and intra-arterially as a 2 per cent solution. The procedure in the third experiment included the intravenous and intra-arterial administration of isotonic solutions of the chlorides of radio-potassium and radio-sodium, the intra-arterial administration of radio-rubidium chloride in isotonic solution, and the intra-arterial administration of radium emanation dissolved in isotonic sodium chloride solution. In the last experiment, isotonic solutions of the chlorides of radio-potassium and radio-rubidium were used. The radio-potassium was administered intravenously and intra-arterially, and the radio-rubidium was given intra-arterially. A solution of the corresponding natural element was given either before or after each radio-element was administered; in the case of the radium emanation an isotonic solution of sodium chloride was used as the control. Each control solution was given by the same channel of administration and in the same concentration and amount as

were the test solutions. The quantity of salt given in each injection varied from $2.5 \times 10^{-5}$ to $10^{-4}$ mols per kgm. of body weight.

RESULTS. No changes in the pulse, respiration, and blood pressure were observed that could be interpreted as being due to the radioactivity of the radio-potassium, radio-rubidium, radio-sodium, and radium emanation. The electrocardiographic tracings, which were taken in the first two experiments, showed no difference of effect between ordinary and activated potassium. The responses to the two varieties of potassium and also of rubidium were identical following their intra-arterial administration, and likewise the action of the natural and activated potassium was the same when given intravenously. The radio-sodium and radium emanation produced no detectable changes in blood pressure, pulse, and respiration when the former was administered intravenously and intra-arterially, and the latter intravenously.

The average of $\beta$-particles emitted per second for the amounts of natural potassium and rubidium, radium emanation, and the activated potassium,

TABLE 1

| ELEMENT | HALF-LIFE | RADIATION | BETA PARTICLES PER SEC. |
|---|---|---|---|
| $K^{40}$ | $1.8 \times 10^{9}$ years | $\beta\gamma$ | 0.03 |
| $Rb^{87}$ | $1.2 \times 10^{11}$ years | $\beta\gamma$ | 3.0 |
| $Na^{23}$ | | | |
| $Rn^{222}$ | 3.8 days | $\alpha\beta\gamma$ | 1,000,000* |
| $K^{42}$ | 12.4 hours | $\beta\gamma$ | 600,000 |
| $Rb^{88 \text{ or } 86}$ | 18.0 days | $\beta\gamma$ | 200,000 |
| $Na^{24}$ | 14.8 hours | $\beta\gamma$ | 1,800,000 |

* There are also emitted 1,500,000$\alpha$ particles per second.

rubidium, and sodium administered per kilogram of body weight, is given in table 1. The values given for the natural potassium and rubidium correspond to the amount of salt which was equivalent in weight to the most strongly radioactive samples of the radio-potassium and radio-rubidium that were administered. In addition to these figures, the table includes the half-life periods and the types of radiations emitted by the substances used in these experiments. The first four isotopes listed in the table are naturally occurring, and the last three are the artificial radioactive isotopes.

DISCUSSION. From table 1 it will be seen that with dosages of equivalent weight of potassium the $\beta$-ray activity was 20,000,000 fold greater for the radio-potassium than for the natural potassium, and in the case of the radio-rubidium the factor was 70,000 as compared with the natural rubidium. In spite of these enormous differences, the physiological action was no different for the activated elements than for their corresponding natural elements. When the radio-sodium was used no physiological

effects were observed, although the $\beta$-ray activity was 60,000,000 times greater than that of the natural potassium. Likewise the radium emanation was physiologically inert although the factor of $\beta$-ray activity was 30,000,000 times greater than that of the natural potassium.

From this information it is apparent that the physiological properties of potassium and rubidium are not due to their radioactivity, and that with the strength of the artificially radioactive samples used there were no toxic manifestations. Furthermore, radioactivity is without demonstrable physiological action when used by the methods described and when administered in the comparatively high intensities available for these experiments.

## SUMMARY

1. The physiological action of natural potassium and rubidium has been compared to that of radio-potassium and radio-rubidium when administered in equivalent concentrations, and no differences in effects have been observed that were due to the induced radioactivity.

2. No detectable physiological effects were noted following the administration of radio-sodium and radium emanation.

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